Case study-454 NAME(s)_____.

I have not given or received any help except from the instructor. Signature_____.

Take Home Exam 1-Part 1Case Study:

For the following case study, write concise answers to the questions and number them. They must be word processed. You will certainly have to use resources other than Stryer, including OMIM to help out.

Introduction

Diabetes mellitus affects 1–2% of people in many populations and is closer to 5% in the USA; its incidence is rising. It is probably the third biggest killer after heart disease and cancer. The account of the disease by Aretaeus of Cappadocia in the 2nd Century AD is highly descriptive:

> Diabetes is a remarkable disorder. It consists of a moist and cold wasting of the flesh and limbs into urine. The disease is chronic in character and is slowly engendered, though the patient does not survive long when it is completely established, for the marasmus produced is rapid, and death speedy.

The polyuria that is characteristic of diabetes led to the origin of the name, from the Greek word meaning 'a siphon'. The Latin appendage 'mellitus' (meaning honeyed) was added by physicians in the 17th Century and relates to the sweet taste of the urine and serum of diabetics. These days, since the isolation of insulin in 1921 by Frederick Banting and Charles Best, diabetes can be controlled. However, the life expectancy of sufferers is probably reduced by one-third, on average, with long-term complications such as renal and cardiovascular disease developing. Diabetes is also the major cause of blindness in many countries. Not all diabetics require insulin to survive. Insulin-dependent (or type I) diabetics represent less than 20% of the total. The remainder (non-insulin-dependent or type II diabetics) are more resistant to the effects of insulin than non-diabetics and can generally be treated by dietary control together with the use of oral hypoglycaemic agents such as sulphonylureas.

As shown in Figure 18.1, insulin is a polypeptide hormone consisting of two disulphide-linked chains, the A chain of 21 amino acids and the B chain of 30 amino acids. Insulin was the first protein to be completely sequenced and the first to be synthesized chemically. It is synthesized in the β-cells of the pancreatic islets as a single chain precursor polypeptide, proinsulin, which is proteolytically cleaved at pairs of basic residues to generate the active hormone by one or more serine proteinases resembling the bacterial enzyme subtilisin. Insulin exerts its actions through a cell-surface receptor which is one of the family of protein tyrosine kinase receptors showing similarity with the epidermal growth factor receptor and the src family of oncogene products. The receptor, like insulin itself, is composed of two types of subunits, α and β, initially synthesized as a single $\alpha\beta$ precursor polypeptide and assembled as a functionally active tetramer, $\alpha_2\beta_2$. The α subunit (M_r 135 000) is the insulin-binding subunit whereas the transmembrane β subunit (M_r 90 000) possesses the tyrosine kinase activity which mediates the intracellular signalling.

To understand the metabolic disturbances in diabetes requires an understanding of nutrient homeostasis and the anabolic role of insulin. Insulin promotes the rapid uptake of nutrients such as glucose and some amino acids into tissues and its absence has often been likened to 'starvation in the midst of plenty'. The present problem focuses on the molecular basis for one severe and inherited form of insulin resistance.

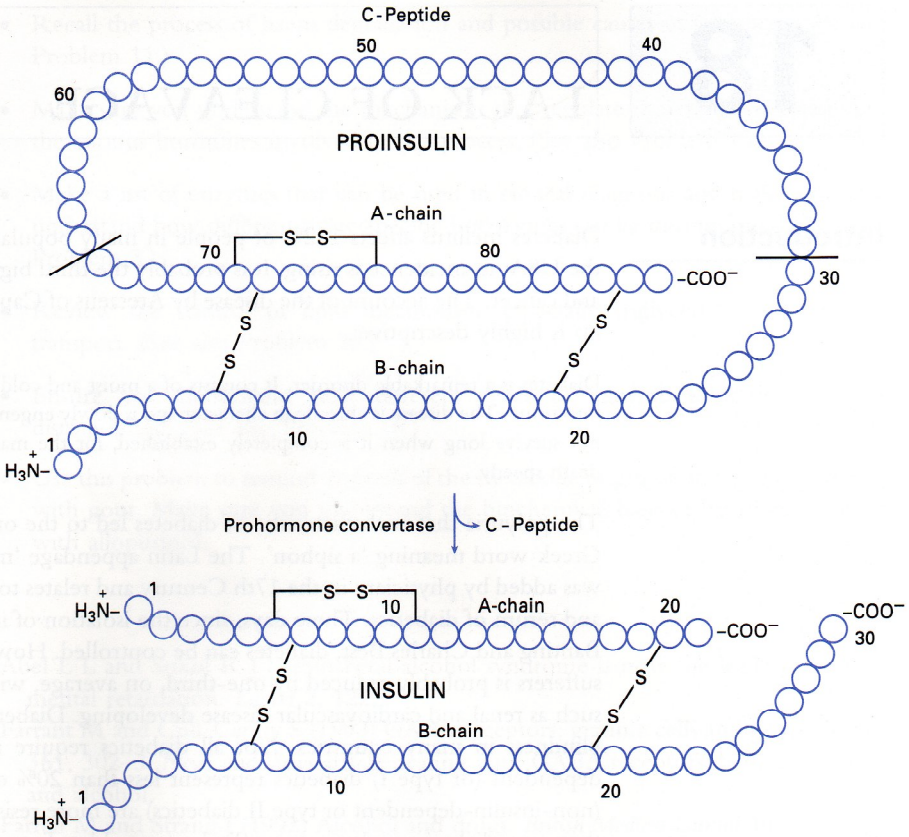


Figure 18.1. Diagrammatic representation of human proinsulin and insulin. Adapted from Devlin (1992) *Textbook of Biochemistry with Clinical Correlations* (3rd edn). Wiley-Liss, New York.

The Problem

A 23-year-old Chinese woman (Suzie Q.) had been diagnosed as diabetic at the age of seven. She had severe hyperinsulinaemia and exhibited many of the typical features of major insulin resistance including primary amenorrhoea, acanthosis nigricans (a hyperpigmented and hyperkeratotic skin rash), hirsutism and virilisation. Table 18.1 indicates the severity of some of the metabolic changes. Suzie also exhibited additional symptoms not normally associated with insulin resistance including mental retardation, short stature and dental dysplasia.

In order to investigate the molecular mechanism underlying Suzie's insulin resistance, cultured fibroblasts from Suzie were compared with those from normal subjects in their ability to bind [^{125}I]insulin (Figure 18.2a). In addition, the ability of insulin to stimulate the uptake of the non-metabolisable sugar, 2-deoxyglucose (2-DOG), into the cultured fibroblasts was examined (Figure 18.2b).

To characterise further Suzie's insulin receptors, a sample of her fibroblasts and those of a control subject were separately treated with [^{125}I]insulin together with a cross-linking reagent to couple bound insulin covalently to its receptors. The cells were then solubilised and the insulin receptors immunoprecipitated with an antibody directed against the human insulin receptor. The precipitate was then subjected to polyacrylamide gel electrophoresis in the presence of the anionic detergent sodium

Table 18.1. Changes of blood insulin and glucose concentrations in Suzie Q.

	Suzie Q.	Normal range
Fasting blood insulin (pM)	2120	20–100
Blood insulin after oral glucose loading (pM)	7120	144–502
Fasting blood glucose (mM)	7–12	3.5–5.5

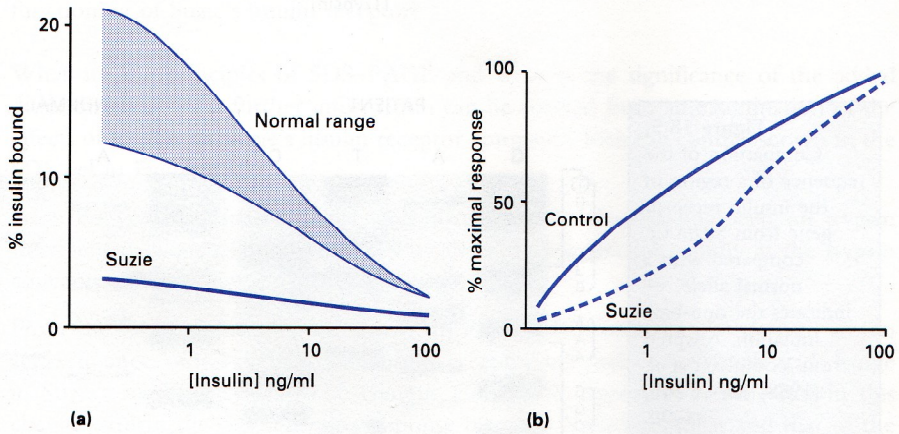


Figure 18.2. (a) Insulin binding to fibroblasts from Suzie Q. and control subjects. The assay measures the ability of increasing concentrations of unlabelled insulin to displace a limiting amount of [^{125}I]insulin. (b) Effects of insulin on the uptake of 2-DOG into fibroblasts from Suzie Q. and a control.

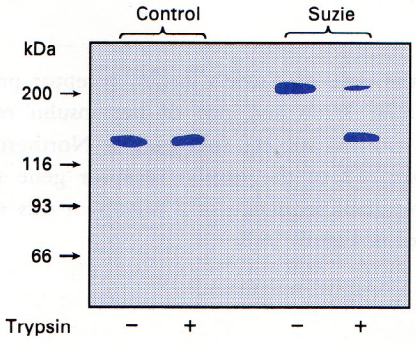


Figure 18.3. Autoradiograph of SDS-PAGE gel showing radiolabelled polypeptides.

dodecyl sulphate (SDS–PAGE); a thiol compound (dithiothreitol) was also included. The same experiment was also performed after brief treatment of the cells with a low concentration of trypsin. An autoradiograph of the SDS-PAGE gel is shown in Figure 18.3. Next, the effect of trypsin on the binding of [^{125}I]insulin to Suzie's fibroblasts was compared with those of a control. Marked differences were seen as shown in Figure 18.4.

Figure 18.4. Effect of *trypsin on insulin* binding to Suzie Q.'s fibroblasts compared with a control.

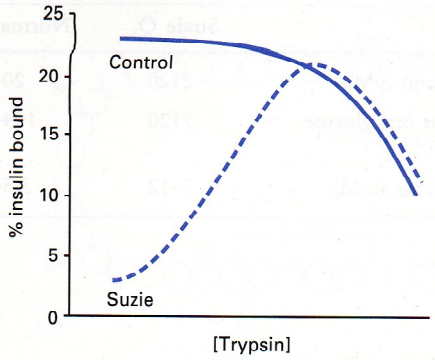
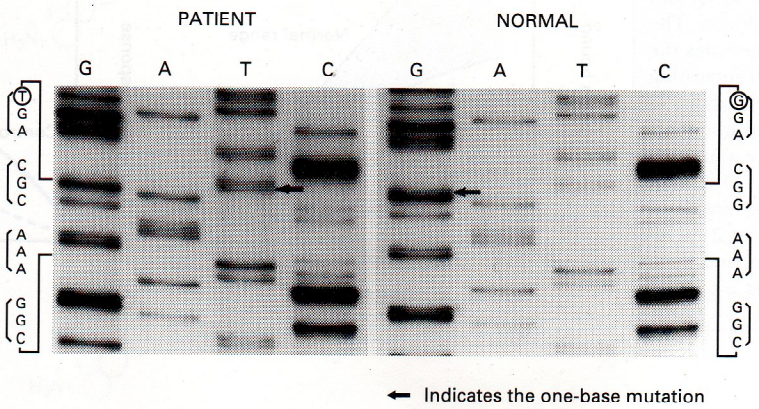


Figure 18.5. Comparison of the sequence of a region of the insulin receptor gene from Suzie Q. compared with a normal allele. ← indicates the one-base mutation.



In contrast to the data on Suzie's insulin receptor protein, no differences were found in the cellular levels and size of her insulin receptor mRNA transcripts compared with a normal subject following a Northern blot analysis. In a more detailed study, exon 12 of the insulin receptor gene (275 bp) was isolated and sequenced. The resultant sequence of a region of this exon is compared between Suzie and a control in Figure 18.5.

Questions. Answer completely (typed!) but concisely.

1) What are the distinctions between Type I and Type II diabetes? How is diabetes diagnosed?
2) What specifically do the data in 18.2a and 18.2b tell you about the function of Suzie's receptor?
3) What does the PAGE gel in 18.3 specifically tell you about Suzie's receptor? What is the significance of the trypsin experiment?
4) How do you explain the initial large increase in insulin binding from trypsin treatment of Suzie's fibroblasts and why does binding rapidly decrease as [trypsin] increases (18.4)?
5) From the sequence in 18.5 use a genetic code table to deduce the amino acid substitution (if any) in Suzie's receptor. How does this result explain the previous data point by point and the differences in the response of Suzie's receptor as compared to the control.
6) Use OMIM to find:
   a) The name or OMIM number of the disorder
   b) Name the specific allele associated with Suzie's disorder (also give amino acid number and substitution).
   c) What chromosome is it on?
   d) Give another KNOWN ***specific*** insulin receptor mutation which causes a similar disorder (give amino acid number and substitution).