This defect is not generalized, as glycogen metabolism responds normally to vaso-
pressin (Hems & Ma, 1976) and does not involve the adenylate cyclase system, both
because vasopressin does not act via this mechanism (Kirk & Hems, 1974) and because
glucagon acts normally on glycogen metabolism in genetically obese mice (G. Y. Ma &
D. A. Hems, unpublished work).

Conclusion

It is still axiomatic that the liver constitutes the main cross-roads for the processes of
interorgan lipid metabolism, i.e. those processes designed to serve the whole animal as
well as individual organ needs. Despite the prime importance of this role, knowledge of
mechanisms of regulation of hepatic fat metabolism is derisory. The preceding account
has been designed to highlight this state of affairs.

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Regulation of Liver Lipid Metabolism in Experimental Obesity

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The physiopathology of experimental obesity, studied in several animal models, has been
reviewed by Assimacopoulos-Jeannet & Jeanrenaud (1976). We summarize here data
obtained with livers from three types of obese animals, the obese–hyperglycaemic
(ob/ob) mouse, the albino rat made obese by electrolytic lesions of the hypothalamus
and the albino mouse made obese by chemical lesions of the hypothalamus.

Materials and methods

Animals used in these experiments were as follows: (a) 8–12-week-old male obese–
hyperglycaemic (C57 BL/6J ob/ob) mice and lean controls (C57 BL/6J ++/++); (b)
female Wistar rats weighing between 180 and 200g; (c) Swiss albino male mice that were
either 9 or 20 weeks old. All animals were fed ad libitum with laboratory chow. In rats,
lesions of the hypothalamus were produced by stereotaxically guided electrolysis,
successful lesions of the ventromedial hypothalamus area being subsequently controlled
histologically. Hypothalamic-lesioned and unoperated control rats were used for liver
perfusion 10 days after the operative day (Karakash et al., 1977). In mice, lesions of the
hypothalamus were obtained by a single injection of gold thioglucose (150–350mg/kg

1977
body wt. intraperitoneally) (Le Marchand et al., 1977b). Gold thioglucose-injected obese mice were used at either 9 weeks (short-term obesity) or 20 weeks of age (long-term obesity). Livers were perfused in situ as described previously (Assimacopoulos-Jeannet et al., 1973; Le Marchand et al., 1973). The addition of \( \text{H}_2\text{O} \) to the perfusion medium permitted the measurement of lipogenesis and the output of newly synthesized (i.e. labelled) triacylglycerol via \( \text{H} \) incorporation from \( \text{H}_2\text{O} \) into lipids (Jungas, 1968). Samples of perfusate were used for the determination of glucose, triacylglycerol, ketone bodies and urea (Karakash et al., 1977). Supernatants of liver homogenates were used for the determination of acetyl-CoA carboxylase activity and fatty acid synthetase (Halestrap & Denton, 1973). For the measurement of glucose-induced insulin secretion \textit{in vivo}, blood samples were withdrawn from anaesthetized animals at various intervals after the rapid injection of glucose (0.5–0.6g/kg body wt.). Plasma immunoreactive insulin was measured by the charcoal-coated immunoassay (Herbert et al., 1973). Some obese–hyperglycaemic mice were made insulin-deficient, 8–10 days before the experiment, by intraperitoneal injection of 200mg of streptozotocin/kg (Assimacopoulos-Jeannet et al., 1974; Karakash et al., 1976) or by injection of anti-insulin serum (0.1 ml neutralizing capacity of 2.16i.u./ml) into a tail vein 2h before the experiment (Karakash et al., 1976). Some hypothalamic-lesioned rats were made acutely insulin-deficient by intravenous injection of 1ml of the above-mentioned anti-insulin serum. Insulin clearance of lean and obese–hyperglycaemic \textit{ob/ob} mice was determined by measuring immunoreactive insulin in the perfusate before and after a single passage through the liver. Calculations were made with individually measured flow rates, wet liver weights and insulin concentrations present in the perfusate entering and leaving the liver. The difference between insulin entering and insulin leaving the liver was related to liver weight and represented the actual removal of insulin by the organ (Karakash et al., 1976). Specific binding of insulin to hepatic plasma membrane was measured by using mono-\textsuperscript{125}I-labelled insulin and plasma membranes purified from livers (Neville, 1968).

\textbf{Results}

\textit{Liver metabolism in obese–hyperglycaemic (ob/ob) mice.} Increased fat deposition can be the result of increased lipogenesis \textit{in situ}, increased uptake of triacylglycerol circulating as very-low-density lipoproteins (VLD lipoproteins) or a combination of both processes (Assimacopoulos-Jeannet & Jeanrenaud, 1976). Owing to this, it was decided to investigate in these mice the metabolism of the organ principally responsible for the production of VLD lipoproteins, i.e. the liver. Perfused livers from untreated \textit{ob/ob} mice (insulinaemia of about 7ng/ml) had lipogenic and triacylglycerol-secretion rates that were about twice those of controls, whereas ketogenesis from exogenous fatty acids was markedly lower and hepatic triacylglycerol content markedly higher than normal (Assimacopoulos-Jeannet et al., 1974). These abnormalities were apparently related to hyperinsulinaemia, since when the latter was lowered by streptozotocin treatment of the \textit{ob/ob} mice before liver perfusion, they were restored to normal (Assimacopoulos-Jeannet et al., 1974). Such normalization of hepatic metabolism was accompanied by that of the activity of both acetyl-CoA carboxylase and fatty acid synthetase which, in livers from untreated \textit{ob/ob} mice, were markedly higher than in controls. The increased hepatic lipogenesis of the \textit{ob/ob} mice could also be returned to normal when hyperinsulinaemia of these mice was acutely 'neutralized' by the administration of anti-insulin serum 2h before the experiment (Assimacopoulos-Jeannet et al., 1977). Moreover, although insulin added to the perfusate (1000\textmu i.u./ml) failed to influence lipogenesis in livers from untreated obese–hyperglycaemic mice, it was effective when added to livers obtained from obese mice treated with anti-insulin serum (Assimacopoulos-Jeannet et al., 1977). The latter observations, as well as those mentioned above, suggested that the hepatic parenchyma of the \textit{ob/ob} mice was in a state of insulin overstimulation owing to hyperinsulinaemia, resulting in both increased lipogenesis and decreased ketogenesis.

\textit{Liver metabolism of rats with electrolytic lesions of the ventromedial hypothalamus.} In
another series of experiments, the likely causal influence of hyperinsulinaemia on the hepatic disorders of experimental obesity was further substantiated. Use was made, in this series of experiments, of the fact that the electrolytic lesions of the ventromedial hypothalamus area in rats results in hyperphagia and hyperinsulinaemia (Karakash et al., 1977). Food intake of rats with ventromedial-hypothalamus lesions was subsequently prevented by placing them on a restricted diet that matched that of unoperated controls. These non-hyperphagic rats with ventromedial-hypothalamus lesions were characterized by an increased basal and glucose-induced hyperinsulinaemia. Perfused livers from such food-restricted rats with ventromedial-hypothalamus lesions released less glucose into the perfusate than controls, whereas urea production and secretion of unlabelled triacylglycerol were increased. Although fatty acid uptake by livers was, after the addition of albumin-bound oleate, the same in controls and in rats with ventromedial-hypothalamus lesions, hepatic ketogenesis was much smaller in livers of the latter. On addition of various substrates to the perfusate (e.g. amino acids, lactate, glucose) lipogenesis and rates of secretion of newly synthesized triacylglycerol were always highest in livers from food-restricted ventromedial-hypothalamus-lesioned rats. This was in keeping with the observation that, under the same experimental conditions, both initial and total acetyl-CoA carboxylase, as well as fatty acid synthetase were greater in rats with ventromedial hypothalamus lesions than in unoperated controls. Most of the above-mentioned hepatic abnormalities of lesioned rats were restored towards normal either by starvation (which was accompanied by the disappearance of the excessive insulin secretion) or by the administration of anti-insulin serum before perfusion. On the basis of these experiments it was concluded that, as in the livers from ob/ob mice, there seemed to be a relationship between prevailing hyperinsulinaemia and the hepatic metabolic abnormalities that followed lesions of the ventromedial hypothalamus area. These abnormalities appear to be characterized by a shift from fatty acid oxidation to fatty acid synthesis and esterification. The enhanced urea production in livers from hypothalamic-lesioned rats might, in addition, reflect a higher degree of deamination of amino acids together with their diversion away from protein synthesis and towards fatty acid synthesis (Karakash et al., 1977; Holm et al., 1973).

Liver metabolism of mice with chemical lesions of the ventromedial hypothalamus. Mice were treated with gold thioglucose, a sugar which is taken up, in particular, by the cells of the ventromedial hypothalamus area, where it produces necrotic lesions responsible for the subsequent development of hyperphagia and hyperinsulinaemia (Le Marchand et al., 1977b). It was observed that lipogenesis and secretion of newly synthesized triacylglycerol were nearly doubled in livers from both groups of gold thioglucose-injected obese mice tested, i.e. in short- or long-term obesity, when compared with their respective controls. Insulin administration to lean mice in vivo resulted in a marked stimulation of hepatic lipogenesis. In contrast, although basal lipogenesis (i.e. in the absence of insulin administration) was much greater in both groups of gold thioglucose-injected obese mice than in control mice (reaching values that were actually similar to those seen in insulin-treated controls), insulin administration to these obese mice completely failed to stimulate this process any further. These results were consistent with the concept of a maximal stimulation of hepatic lipid metabolism in gold thioglucose-injected obese mice, presumably owing to their hyperinsulinaemia (Le Marchand et al., 1977b).

Hormone binding to liver membranes in normal and obese mice. The binding of insulin to plasma membranes of livers from ob/ob mice was very low (Le Marchand et al., 1977a). Insulin binding markedly increased in liver membranes of ob/ob mice when the latter were subjected to 12 days of starvation, a dietary manipulation that brought their insulinaemia from a 'fed' value of 25 ng/ml to 0.5 ng/ml. In liver membranes obtained from starved ob/ob mice, insulin binding was even higher than that observed in membranes from fed lean mice. The binding of insulin was also markedly increased in liver membranes obtained from streptozotocin-treated ob/ob mice when compared with that observed in untreated obese animals. The greatest degree of increase in insulin binding was observed in membrane from mice in which streptozotocin treatment had been most
effective in lowering hyperinsulinaemia (Le Marchand et al., 1977a). Insulin binding to liver membranes was slightly decreased in gold thioglucose-injected obese mice in which obesity was of short duration, and markedly decreased when obesity was long-term (Le Marchand et al., 1977b). Thus the progressive decrease in insulin-binding capacity of liver membranes was related to the duration of obesity caused by gold thioglucose and paralleled by a progressive increase in these obese mice, in both basal and glucose-induced hyperinsulinaemia. These data indicated that in spontaneous (ob/ob mice), as well as in the experimentally produced, obesity (gold thioglucose-injected mice), there was a marked decrease in insulin binding to liver that appears to be due to an actual decrease in insulin receptor number per mg of membrane protein. However, this decreased insulin receptor number did not prevent overstimulation of liver lipid metabolism as mentioned above.

Hepatic clearance of insulin and hyperinsulinaemia. The liver has been shown to be important in determining the amount of insulin reaching the peripheral blood, since it 'removes' significant quantities of insulin (Field, 1972). The extent of insulin removal by the liver has been found to be greater when the amount of the hormone reaching the portal vein was lowest (McCarroll & Buchanan, 1973). On the basis of these experiments, it was hypothesized that in a hyperinsulinaemic state, such as that of the ob/ob mice, the liver might be defective in its handling of insulin. It was observed (Karakash et al., 1976) that the absolute amount of insulin removed by the liver increased with increasing concentrations of the hormone infused into the portal vein, until the process became saturated. This was true for livers of both lean and obese—hyperglycaemic mice. However, the striking observation made was that the removal of the hormone by livers of obese—hyperglycaemic mice was always less than that of lean controls (Karakash et al., 1976). Consequently, at each insulin concentration tested, the percentage of insulin removed by the liver was always lowest in livers obtained from obese—hyperglycaemic mice. This abnormality was secondary to hyperinsulinaemia since, when hyperinsulinaemia of the ob/ob mice was decreased (starvation, streptozotocin treatment, administration of anti-insulin serum), the removal was normalized. Although the hepatic removal of insulin is ill-defined in its nature, these data suggest that in hyperinsulinaemic states the observed decrease in hepatic insulin 'clearance' may worsen hyperinsulinaemia. This would help to reinforce the disturbances of lipid metabolism noted above.

Discussion and conclusions

Hyperinsulinaemia of obese animals appears to be responsible for most of the abnormalities of liver metabolism noted above, since they decrease or disappear when hyperinsulinaemia is decreased or suppressed. The close resemblance of liver abnormalities in spontaneously obese mice (ob/ob type) and in experimentally produced obesity (lesions of the ventromedial hypothalamus) further suggests that the common pathological trait is likely to be hyperinsulinaemia. In the experimentally produced obesity that we have investigated, hyperinsulinaemia was clearly of hypothalamic origin. In spontaneous syndromes, hyperinsulinaemia could also be hypothalamic in its origin (Coleman, 1973), although a primary B-cell dysfunction has been suggested (Strautz, 1970). One should note that in both spontaneously obese ob/ob as well as in gold thioglucose-injected obese mice, overstimulation of liver lipogenesis occurred, despite a marked decrease in insulin-binding capacity of isolated membranes. This observation is in agreement with the concept that maximal effects of insulin can be achieved when only a small fraction of the total receptor is occupied (spare-receptor theory). The observation that in these two types of obesities some tissues (e.g. the liver) are over-stimulated, whereas others (e.g. muscles) are not (Cuendet et al., 1976; Le Marchand et al., 1977a,b) may explain why these animals become both obese and hyperglycaemic. Indeed, it appears that lasting hyperinsulinaemia includes a generalized decrease in insulin binding to plasma membranes, possibly without (liver) or with (adipose tissue, muscle) additional intracellular metabolic abnormalities (Czech, 1976; Freychet, 1976; Olefsky, 1976; Le Marchand et al., 1977a). The latter would interfere, by still ill-defined mechanisms, with normal glucose utilization and prevent overstimulation of some tissues.
Regulation of the Conversion of Glucose into Fat in White Adipose Tissue by Insulin

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Within a minute or so of exposure to physiological concentrations of insulin, the rate of conversion of glucose into fat in rat epididymal adipose tissue is greatly increased. This increase appears to involve not only activation of glucose transport (Crofford & Renold, 1965; Vinten et al., 1976) but also of pyruvate dehydrogenase (Jungas, 1971; Denton et al., 1971; Weiss et al., 1971, 1974; Coore et al., 1971; Martin et al., 1972; Severson et al., 1974, 1976; Stansbie et al., 1976a, b; Taylor et al., 1973; Taylor & Jungas, 1974), acetyl-CoA carboxylase (Halestrap & Denton, 1973, 1974) and possibly one or more steps in the processes of fatty acid esterification (Jason et al., 1976; Sooranna & Saggerson, 1976). Both pyruvate dehydrogenase and acetyl-CoA carboxylase exist in interconvertible forms; after exposure to insulin the proportion of both enzymes in their respective active forms is increased 2-3-fold (Table 1). These parallel activations appear to offer a satisfactory explanation of the preferential conversion of glucose carbon into fatty acid which is so characteristic of the response of rat adipose tissue to insulin. This article summarizes some of our recent studies relevant to the...