Hepatic protein turnover in goldthioglucose-induced obesity

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Summary. Goldthioglucose obese mice fail to regulate hepatic protein turnover in response to variable energy intake in contrast to lean mice.

It has been suggested that dietary-induced thermogenesis and cold-induced thermogenesis may have the same biochemical basis[1-3]. Recently, evidence has been presented that both protein turnover[1] and brown fat metabolism[2] are enhanced in response to increased food intake and furthermore that both of these responses are defective in the genetically obese *ob/ob* mouse[1,4,5]. A defective dietaryinduced thermogenesis could then either be a basis of the obesity or could be secondary to the obese state but a contributory factor to the maintenance of that obese state. In this paper, we report studies of the regulation of hepatic protein turnover in mice made obese by goldthioglucose (GTG), which suggests that an impairment in the regulation of hepatic protein turnover may be secondary to the obese state.

Methods. 4-week-old lean male mice were injected with 1.6 mg/g b.wt GTG (Sigma Chemical Co. Ltd) and allowed to gain weight until the static phase of obesity was reached (16–17 weeks of age). 20 GTG obese mice were then adapted over a 3-week period to variable food intakes and to a 4-h meal feeding regime as previously described[1].

Digestible energy intakes were assessed from the food intake and faecal energy losses[1]. Hepatic protein turnover was measured during the following 2 weeks while maintaining the mice on their feeding regimes. The 4-h feeding regime was used to alleviate any differences in feeding pattern which might occur in animals given a restricted food intake.

Mice were injected i.p. with 100 µCi DL-[2-^{3}H]-glutamic acid (sp. act. 4.5 Ci/mmole) (Radiochemical Centre, Amersham, Bucks.). After 3, 6, 9, 12 and 15 days, 4 mice from each dietary group were sacrificed and both the total and specific activities of ^{3}H-labelled protein in the liver were determined to calculate the half-lives of protein synthesis and catabolism[1]. Protein turnover was then calculated:

$$\text{Protein turnover (mg/d)} = \frac{\text{total tissue protein} \times \ln 2}{\text{rate limiting half-life}}$$

Results and discussion. After adaptation to differing feeding regimes, the body weights of all GTG mice were stable with the exception of GTG mice fed only 60% of the ad libitum food intake whose weight continued to fall slowly (fig. 1). Hepatic protein turnover of lean mice was shown previously to have a sigmoidal relationship to energy intake[1]. In contrast, there was little change in hepatic protein turnover in the GTG obese mice over a wide range of energy intakes (fig. 2). Indeed, the relationship of protein turnover to dietary intake of GTG obeses mice paralleled that which we have previously reported in genetically obese *ob/ob* mice and which is also shown for reference in fig. 2. The relative decrease in the rate of protein turnover in the GTG obese mice probably reflected their greater age since it was necessary to use older GTG obese mice in order to obtain a similar degree of obesity to the ob/ob mice. Protein turnover is known to decrease with age[6].

The abnormal regulation of hepatic protein turnover in 2 forms of obesity, one of genetic origin *(ob/ob)* and the other of a dietary-induced form (GTG) suggests that the obese state and its associated endocrine changes may be responsible for these changes. The fractional catabolic rate of muscle myofibrillar protein is enhanced in obese *(ob/ob)* mice[7]. This, in the presence of normal synthesis, results in a fall in muscle tissue mass. Thus, it appears that the control of protein turnover may be altered in the muscle as well as in the liver of obese mice.

Thyroid hormones are known to regulate protein turnover[8]. Thyroid hormone secretion appears to be normal in both GTG and obese *(ob/ob)* mice[9]. However, a loss of triiodothyronine (T_3) receptors in hepatic and spleen nuclei of *ob/ob* mice has been reported[10]. In addition, the [$Na^+ + K^+$]-ATPase activity of *ob/ob* mice is less responsive to thyroid hormones than that of lean or GTG obese mice[11], due to an

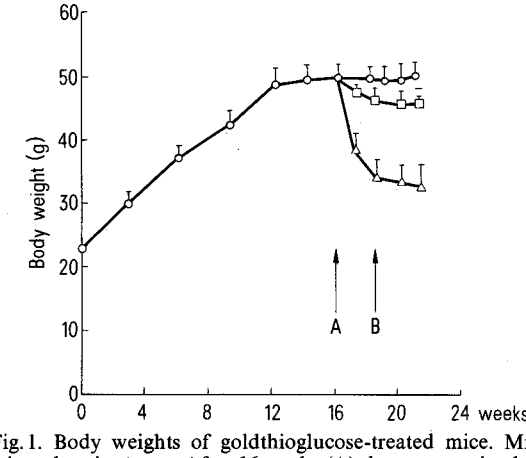

Fig. 1. Body weights of goldthioglucose-treated mice. Mice were injected at time zero. After 16 weeks (A) they were trained to eat on a 4-h feeding regime either ad libitum or at either 80% or 60% of the ad libitum intake. After a further 3 weeks (B) all animals were injected with 100 µCi DL-[2-^{3}H]-glutamic acid for measurement of protein turnover.

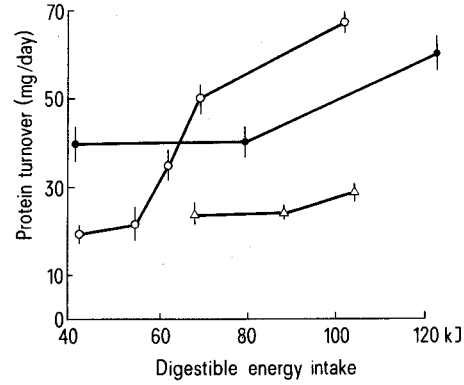

Fig. 2. The effect of food intake on hepatic protein turnover in goldthioglucose obese mice. The protocol is described in the legend to fig. 1. Protein turnover for lean and *ob/ob* mice have been previously published[1] but are given for reference. GTG (△—△), *ob/ob* (●—●) and lean *ob/*? (○—○).

altered relationship between enzyme units and activity[12] as the induction of enzyme units appears to be normal[13]. The data suggests that thyroid hormone metabolism may be altered in *ob/ob* mice but be normal in GTG mice. Thus, it seems unlikely that the common defect in hepatic protein turnover in these 2 obese species is related to thyroid hormones, and the underlying mechanisms await further investigation.

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Inability of dithiols to cause activation of *Limulus* endotoxin sensitive procoagulase

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Summary. Using a chromogenic substrate it has been shown that the endotoxin sensitive procoagulase of *Limulus* lysate is not activated by dithiols. Increased turbidimetric readings in the presence of dithiols would therefore appear to be nonspecific.

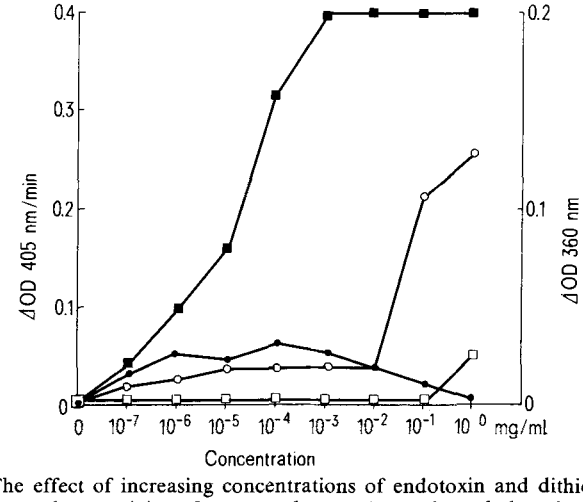
The *Limulus* amoebocyte lysate (LAL) method is currently used for the measurement of endotoxin in serum, biological and pharmacological products[1]. The use of the assay has come under some criticism since certain proteolytic enzymes, synthetic polynucleotides and peptidoglycans were found to give presumably false positive results[2-4]. Recently low molecular weight dithiols were reported to simulate endotoxin in the LAL assay at concentrations of 1 µg/ml or greater[5]. In this report we show that this apparent activity is not due to a specific activation of the *Limulus* lysate, as occurs in the presence of endotoxin.

Materials and methods. LAL was obtained from Sigma Chemical Co. and reconstituted in pyrogen free water. The LAL test was carried out according to a variation[6] of the chromogenic substrate method of Iwanaga et al.[7]. To 200 µl of 0.09 M Tris-HCl pH 8.0, 0.035 M $MgCl_2$ was added 150 µl of endotoxin or the dithiol, dithiothreitol (DTT) (Sigma) dissolved in saline, and 20 µl lysate. After 20 min at 37 °C in the LKB 2086 Mk II Reaction Rate Analyzer 50 µl of 1.25 mM chromogenic peptide substrate S2222 (Bz-Ile-Glu(γ-OR)-Gly-Arg-p-nitroanilide-HCl Kabi Vitrum Ltd) was injected and the change in optical density calculated using an LKB 2082 Kinetic Data Processor with fixed time programme. Similar mixtures were also made and incubated but without the addition of S2222, a turbidimetric measurement being made at 360 nm according to Platica et al.[5].

Results. The results (fig.) show a significant increase in turbidity at concentrations of dithiol greater than 10 µg/ml confirming the work of Platica et al.[5]. Much lower turbidity levels were observed by these workers in the presence of endotoxin and in fact in our system the dilution of lysate is such as to cause no increase in turbidity except at the highest concentration of endotoxin.

When the measurements are made using chromogenic substrate, however, endotoxin is shown to cause a rapid generation of enzyme reaching a peak at concentrations greater than 100 ng/ml. Increasing concentrations of DTT cause a much lower generation of enzyme reaching a peak approximately 10 times less than that observed with endotoxin. At concentrations of DTT at which a large increase in turbidity is observed there is a marked inhibition of enzyme activity which is total at 1 mg/ml.

Discussion. The highly sensitive endotoxin assay using amoebocyte lysate from the haemolymph of the *Limulus* crab rests on the observation that the solution gelates in the presence of endotoxin and using suitable conditions the rate of formation of a gel is dependent on endotoxin



The effect of increasing concentrations of endotoxin and dithiols upon the reactivity of *Limulus* lysate. Squared symbols refer to observations made in the presence of endotoxin, circular symbols to those made in the presence to DTT. Closed symbols refer to the change in absorbance at 405 nm and open symbols to the turbidimetric readings at 360 nm.