SOME METABOLIC ASPECTS OF DIETARY THERMOGENESIS

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Nutrition of the University of Surrey.

March 1978.
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SUMMARY

The effect of varying exercise levels on the thermic effect of a mixed nutrient meal was measured in normal young adults. Although exercise was found to potentiate the thermic effect in three subjects there was no consistent effect of exercise in nine other subjects. Blood glucose, serum non-esterified fatty acid, serum glycerol and serum insulin levels were observed before and after the meal.

Rate of weight gain, together with metabolic rate before and after a glucose meal, were studied in a group of fifteen female anorexia nervosa patients as their weight was being restored to normal levels. The previously obese anorectic patients gained weight more rapidly, on the same food intake, than those who were of normal weight before their illness began. The increase in resting metabolic rate (as treatment progressed) was less in the previously obese patients, who also showed a tendency for the metabolic rate to increase less after a glucose meal than the patients with no history of obesity. The thermic effect of glucose was greater in patients with anorexia nervosa than in a comparable control group of six female students.

Blood glucose, plasma insulin and plasma glucagon levels were observed before and after a glucose meal in the anorexia nervosa patients. Glucose tolerance was normal in newly admitted emaciated patients, but became impaired as patients were refed and gained weight. Late treatment patients also showed delayed insulin peaks, high insulin levels and greater insulin resistance. Glucagon levels were high in some patients.
I would like to thank Professor Vincent Marks for his constant encouragement and helpful advice.

My thanks go too to Gill Farrant, Anthea Cridland and Mary McKie who worked with me on some of the energy expenditure experiments and all the subjects and patients who made this project possible.

I am grateful to Professor Arthur H. Crisp for giving me free access to his patients and Professor Keith Buchanan for enabling me to use his glucagon assay.

Finally, I would like to thank Valerie Saunders for her skill and patience while typing this thesis.
Note on Units used in Reporting Results

The SI system has not been used in the present thesis for two reasons; (1) when this study was commenced, a firm decision had not been taken to adopt SI units internationally; (2) the units used facilitate comparisons between results obtained in the present studies and those previously reported by others.
**Abbreviations**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACT</td>
<td>Overactive</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
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<tr>
<td>BC</td>
<td>Before Christ</td>
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<tr>
<td>BMR</td>
<td>Basal metabolic rate</td>
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<tr>
<td>BUL</td>
<td>Bulimic</td>
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<tr>
<td>C</td>
<td>Centigrade</td>
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<tr>
<td>cal</td>
<td>Calorie</td>
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<tr>
<td>DUR</td>
<td>Long duration of illness</td>
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<td>et al</td>
<td>Et aliae</td>
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<tr>
<td>FAD</td>
<td>Flavin-adenine dinucleotide</td>
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<td>Fig</td>
<td>Figure</td>
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<td>g</td>
<td>Gramme</td>
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<tr>
<td>GB</td>
<td>Basal glucose</td>
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<td>GLI</td>
<td>Glucagon-like immunoreactivity</td>
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<tr>
<td>GP</td>
<td>Peak glucose</td>
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<td>GPD</td>
<td>Peak glucose above basal</td>
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<tr>
<td>GRT</td>
<td>Glucose 2 hour/1 hour ratio (G120E/G60D)</td>
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<tr>
<td>G60</td>
<td>Mean glucose above origin 0-60 minutes</td>
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<tr>
<td>G60D</td>
<td>Mean glucose above basal 0-60 minutes</td>
</tr>
<tr>
<td>G120D</td>
<td>Mean glucose above basal 0-120 minutes</td>
</tr>
<tr>
<td>G120E</td>
<td>Mean glucose above basal 60-120 minutes</td>
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<tr>
<td>GIP</td>
<td>Gastric inhibitory polypeptide</td>
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<tr>
<td>HP</td>
<td>High protein</td>
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<td>hr</td>
<td>Hour</td>
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<tr>
<td>IB</td>
<td>Basal insulin</td>
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<tr>
<td>IP</td>
<td>Peak insulin</td>
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<tr>
<td>IFD</td>
<td>Peak insulin above basal</td>
</tr>
<tr>
<td>IRG</td>
<td>Immunoactive glucagon</td>
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<tr>
<td>IRT</td>
<td>Insulin 2 hour/1 hour ratio (I120E/I60D)</td>
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<td>I60</td>
<td>Mean insulin above origin 0-60 minutes</td>
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<td>I120</td>
<td>Mean insulin above origin 0-120 minutes</td>
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<td>I60D</td>
<td>Mean insulin above basal 0-60 minutes</td>
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<td>I120D</td>
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<td>I120E</td>
<td>Mean insulin above basal 60-120 minutes</td>
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<tr>
<td>IRP</td>
<td>Insulin-releasing polypeptide</td>
</tr>
<tr>
<td>Kcal</td>
<td>Kilocalorie</td>
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<tr>
<td>Kg</td>
<td>Kilogramme</td>
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<tr>
<td>Kj</td>
<td>Kilojoule</td>
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<tr>
<td>l</td>
<td>Litre</td>
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<tr>
<td>LP</td>
<td>Low protein</td>
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Abbreviations (continued)

m  metre
M  molar
mCi  millicurie
mEq  milliequivalent
mg  milligramme
min  minute
MJ  megajoule
ml  millilitre
mos  months

NAD  nicotinamide adenine dinucleotide
NADH  reduced nicotinamide adenine dinucleotide
NADP  nicotinamide adenine dinucleotide phosphate
NADPH  reduced nicotinamide adenine dinucleotide phosphate
NEFA  non-esterified fatty acid
ng  nanogramme
NO  previously non-obese
NS  not statistically significant

oz  ounce
p  page
p <  probability less than
pg  picogramme
PO  previously obese
PUR  purging

RMR  resting metabolic rate
r.p.m.  revolutions per minute

SDA  specific dynamic action
SE  standard error of the mean

T₃  triiodothyronine
T₄  thyroxine

U  unit

VOM  vomiting

yrs  years

μg  microgramme
μl  microlitre
μM  micromole
μU  microunit

Conventional chemical nomenclature was used.
CHAPTER 1

LITERATURE REVIEW

General historical background
Nomenclature
Duration of the thermic effect of meals
Energy expenditure at rest
The specific dynamic action of nutrients -
  Thirty .... four .... and six
Luxus consumption
Drug induced thermogenesis
The size of the thermic effect of meals
Exercise and dietary thermogenesis
Nutritional status and dietary thermogenesis
  undernutrition - adults
  undernutrition - infants
  anorexia nervosa
  obesity
Biochemical pathways of thermogenesis
  uncoupling of oxidative phosphorylation
  poorly phosphorylating pathways
  energetically wasteful cycles
Hormonal control of thermogenesis
Lavoisier began the investigation of energy metabolism about 150 years ago when he recognised the importance of oxygen and enunciated the principles of combustion both outside and within the body. With the development of human respiration chambers by Atwater and their detailed use by Benedict many fundamental problems concerning human energy expenditure were elucidated. Early in these investigations two interesting phenomena were observed; firstly, the increase in oxygen consumption following meals and secondly, overeating does not necessarily result in an equivalent gain in weight.

Nomenclature

The increased metabolic rate following a meal has been termed SPECIFIC DYNAMIC ACTION and the overeating phenomenon LUXUSCONSUMPTION. The literature, however, is confused and in some cases the term, specific dynamic action, is used when long term measurements of the efficiency of food utilisation have been made. Miller, Mumford & Stock (1967) suggest that, specific dynamic action is related to the composition of food, whereas luxusconsumption is related to nutritional status, and that as both are measured as a thermic response to feeding it is probably better to regard them as different aspects of a general phenomenon called DIETARY INDUCED THERMOGENESIS, although these distinctions made by Miller are useful, I prefer to use the term THERMIC EFFECT OF A MEAL for the short term postprandial increase in metabolic rate and LUXUSCONSUMPTION for the
ability to overeat without weight gain because both the thermic effect of a meal and luxusconsumption may be altered by a variation in nutrient content of the meal or diet:

**Duration of the thermic effect of meals**

It is difficult to indicate when the effect of a specific meal ceases and becomes hidden by some long term variation of metabolic rate. If one is observing the metabolic rate after breakfast it may not return to the premeal value until late evening because of the diurnal rhythm of resting metabolic rate. Frequently the specific dynamic action of nutrients is expressed as a percentage of the energy content of the nutrients consumed. Such a measurement assumes that a total measure of the peak area of the meals thermic effect is made. In many circumstances especially in experiments with man, it is difficult to continue measurements for such long periods. Because of the necessity for the person to be idle, it becomes very tiresome and in some experiments they may become restless with hunger. Murlin, et al. (1936) measured, specific dynamic action, for periods 6 to 10½ hours post prandial to meals of butter and butter and sugar, and found the specific dynamic action of fat to be 4.74% of ingested energy. The experiments of Abel (1943) and Jahn & Strossenreuther (1928) indicate that the specific dynamic action of proteins, or high protein foods, does not fall to within basal (premeal) values within six to eight hours. Glickman, et al. (1948) avoided extremely long experiments by predicting the total specific dynamic action; after fitting a suitable equation to the data for the 6 hour post prandial period they extrapolated the duration and size of the total effect. The total predicted duration of the thermic effect after their high protein meal (993 Kcal, 27% protein, 46% fat and 17% carbohydrate) was 16.54 hours and
Figure 1: Diagrammatic representation of the metabolic rate in man throughout 24 hours.

Figure 1a - normal food intake.
Figures 1b and 1c - hypothetical patterns for metabolic rate during overfeeding.

--- normal food intake --- high food intake

**Figure 1a**

- Breakfast
- Lunch
- Dinner

**Figure 1b**

**Figure 1c**

8 12 16 20 24 4 8 hours
for the high carbohydrate meal (1070 Kcal, 7% protein, 30% fat, 54% carbohydrate) 15.12 hours.

More recently Garrow & Hawes (1972) measured oxygen consumption in man after meals of equal energy value (650 Kcal) but varying nutrient composition. They found the metabolic rate was still elevated 3.5 hours after meals of 7% milk protein and sugar, gelatin, and fat, but had returned to the premeal value only three hours after the sucrose meal. Miller and his colleagues (1967) showed that the duration of the effect was dependent on the energy content of the meal. They reported the thermic responses to meals of normal size, 500–630 Kcal, and to meals approximately twice normal. The metabolic rate was raised for approximately three hours after the small meals and eight hours after the large meals. In some observations on infants recovering from malnutrition, Brooke & Ashworth (1972), found the metabolic rate was still raised three hours after the test meal. The problem may be illustrated in diagrammatic form. Fig. 1a shows a hypothetical, idealised picture of oxygen consumption of man throughout the day. The three peaks represent the thermic effects of each of three meals, and the overall diurnal variation of metabolic rate has been ignored.

The question could be posed - "Is luxury consumption an increase in each peak area as in Fig. 1b which may result in a raised metabolic rate during the daytime between meals because the thermic effects of large meals overlap?" or "Does the plethora of nutrients drive the metabolic rate faster independent of the meal stimulus?" as in Fig. 1c.
Figure 2: Circadian oxygen consumption in man on normal diet, after 15 days overeating, and after 15 days restricted diet.

(adapted from Apfelbaum, et al., 1971).

100% = average oxygen consumption/minute over 24 hours on the normal diet.

---

--- normal diet

overeating (normal diet plus 1500 Kcal/day)

restricted diet (220 Kcal/day)
Apfelbaum, et al. (1971) seem to support the picture seen in Fig. 1c. They give examples of 24 hour oxygen consumption on both an overfeeding regime and an underfeeding regime (Fig. 2). The peaks following the meals are not noticeably greater after 15 days overfeeding but the overall level of metabolism was increased by 12-29%. The basal metabolic rate was also raised on average by 12.5%. Those on the restricted diet had an overall decrease of 12-17% and an average decrease in basal metabolic rate of 14.5%.

Energy expenditure at rest

Traditionally, three components of the energy expenditure of everyday life have been considered; the energy cost of activity, dietary induced thermogenesis and basal metabolic rate. To make comparisons of energy balance between individuals these factors have to be standardised and the traditional response has been to measure basal metabolism in the absence of gross muscular activity, in the post-absorptive state, in the zone of thermal neutrality, with minimal emotional disturbance but while the person is awake, in the absence of any disease or infection and in the "normal" nutritional state. Comparison between individuals and species have been on this basis, but are extremely difficult to evaluate. One problem which arises is how one defines the post-absorptive condition. When does an animal go from the post-absorptive state into short term starvation? For man a time of 12-15 hours after the last meal is the usual time for measuring basal metabolic rate but it is impossible to state whether the equivalent condition for the rat is 3.6 or even 15 hours of food deprivation.
The measurement of basal metabolic rate in man does not represent any occasion of long duration in everyday life and so there is a tendency in the recent literature to refer to resting metabolic rate or so-called standard metabolic rate and the conditions under which it was measured rather than restrict the conditions of experimentation.

Basal metabolism does not vary directly with simple body weight and so another problem is the unit to be used in expressing these measurements. The relationship between energy expenditure body size and composition has been much discussed and a good review of the subject was made by Mitchell (1964). Alternatives that have been used are for the energy expenditure to be expressed per square meter body surface area, or to the three fourths power of the body weight or body weight to the power 0.73. More recently, Miller (1975) suggested that resting and basal metabolic rate requirements should be expressed in gross terms and not related to any parameter of body size because what matters for an individual in energy balance is the total proportion of intake energy that is used in resting metabolic rate and activity. This was also discussed by Garrow (1974) who points out that although for individuals it may be adequate to use gross terms, for comparison of groups of individuals of very different build some of the variation may be reduced by relating energy expenditure to surface area or body weight.

Basal metabolic rate has been shown by very many workers to decrease during undernutrition in both man and animals (Benedict, 1915; Benedict, et al., 1919; Forbes, et al., 1931; Hellberg, 1949; Johnston & Maroney, 1936; Keys, et al., 1950; Apfelbaum, et al., 1971), but there is conflicting evidence for an increase in basal metabolic rate after a period
of overeating or to use Mitchell's phrase surfeit nutrition. Munro (1950); Gulick (1922); Strang, et al. (1935); Miller, et al. (1967) were unable to show any rise in basal metabolic rate but it is quite clear from Apfelbaum (1971) in certain circumstances overfeeding results in a raised basal metabolic rate. As Miller writes in 1973, "In fact the presence or absence of a change in basal metabolic rate depends upon which baseline value one takes", and "... it should not be forgotten that a reduction of basal metabolic rate by 10% which would not be regarded as deviating from normal and which would be within the experimental error of most determinations would be sufficient to double weight every 10 years, all other things being equal!"

Garrow (1974) recalculated the results from Miller's experiments and showed that although the metabolic rate first thing in the morning (basal metabolic rate) was not significantly greater in the overfed group the metabolic rate between midnight and 8 a.m. was 31% higher.

The specific dynamic action of nutrients - Thirty ... four ... and six

For many years the concept of specific dynamic action was closely allied to a belief that protein was the most potent cause of dietary thermogenesis. This originated with Rubner (1902) and his experiments with dogs which demonstrated that when a dog was kept in a thermoneutral environment, in a calorimeter, the heat production varied according to the diet fed, when meat was fed the heat production was greatest, it was less high on fat, still less on sugar, and even less on starvation. Garrow (1973) re-examined Rubner's data in detail and demonstrated that there is considerable uncertainty about the true metabolic effects of single nutrients which can be interpreted from Rubner's classical experiment. For example, the metabolic rate was
Figure 3: Energy expenditure in a dog on various diets. The results of an experiment by Rubner (1902) recalculated from the original experimental data. During the course of the experiment the weight of the dog fell from 5.47 to 4.40 kg. (adapted from Garrow, 1973).
highest after the initial 5 days starvation when some unspecified diet was fed (see Fig. 3).

It was Lusk (1928 & 1931) who popularized Rubner's ideas in a modified form. He suggested the specific dynamic action of fat was less than that of carbohydrate. Both Rubner's and Lusk's results are frequently quoted in a misleading form for which they may have been themselves responsible. Brody (1945) quotes Rubner's definition of specific dynamic action; "if the post-absorptive expense of maintenance of a mature normal animal (dog) at thermal neutrality is 100 cal a day, then if the animal consumes 100 cal in the form of meat, its heat production will increase to 131 cal for the day". Garrow (1973) quoted Lusk 'a 100 cal meal of protein will give rise to a heat production of 130 cal a similar meal of glucose 106 cal and of fat 104 cal'.

If this were indeed the case energy balance would be impossible because there would be a continuous drain on energy reserves in order to pay the tax of specific dynamic action. In fact, if we look once more at Rubner's dog we find it lost weight! Whether this was because of several periods of starvation or the energy cost of specific dynamic action is impossible to verify so long after the event.

Kriss, Forbes and Miller (1934) in experiments on young rats (100 g) reported heat increments of casein, starch and olive oil to be 31.4%, 22.5% and 16.5% of the metabolisable energy of the meal respectively. These values for starch and oil are much higher than the previously reported values, possibly because they were made with reference to
maintenance metabolism as a base. The rats were fed a maintenance ration for a few days, then maintenance food + test food for several days. Rubner, on the other hand, had starved his dog and then fed the test food. From these results it appears that nutritional status affects the specific dynamic action of food.

Lusk (1931) reported differences in the specific dynamic action of specific amino acids with high values for phenylalanine, alanine and glycine whereas glutamic acid, aspartic acid and asparagine were reported to give negative results. Lundsgaard (1931), as well as Kriss (1941) found the specific dynamic action of all amino acids to be the same, and Kriss comments that the important calorigenic factor is the metabolisable energy not the nitrogen (protein) consumed or excreted. However, the idea that protein is the most important factor remained in the literature and in 1964 Krebs proposed a theory to explain the specific dynamic action due to protein. He suggested that the effect represented the additional energy necessary for adenosine triphosphate synthesis when protein, rather than fat or carbohydrate, is the oxidative substrate. Krebs concludes: "Two main factors are responsible for the specific dynamic action, each contributing an approximately equal share. One is the energy requirements of the urea synthesis, the other the wasting of some of the energy of amino acid degradation".

Garrow and Hawes (1972) tested Krebs' hypothesis by comparing the specific dynamic action of a meal of gelatin with an equicaloric meal of sucrose. Gelatin is deficient in tryptophan and cannot be used for protein synthesis when fed alone, thus such a meal should increase urea synthesis and according to Krebs the specific dynamic action should be higher. The
sucrose meal on the other hand should reduce urea synthesis and the specific
dynamic action. Gelatin failed to cause a greater specific dynamic action
than sugar despite a greatly increased urea production and so they concluded
that they failed entirely to obtain results consistent with Krebs' explanation of the mechanism of specific dynamic action.

Despite specific dynamic action being linked mainly with protein it was soon realised by some nutritionists, particularly those working with animals, that the specific dynamic effects of isolated nutrients fed as such have little to do, if anything, with the specific dynamic effects of mixtures of nutrients. Mitchell (1934) stated, "When the nutrients are fed in combination the dynamic effects of the mixtures will be less than the weighted mean of the individual effects, and this decrease will continue as the combinations approach a perfectly balanced combination for the animal under experimentation". The experiments with rats of Forbes, et al. (1935 a & b and 1946 a, b & c) and Hamilton (1939) substantiate this view. Hamilton showed a higher specific dynamic action with diets very high or very low in protein and Forbes and Swift a decrease in the heat production in the order of increasing fat contents of the diets. Other experiments in man which suggest protein is not the main determinant of the size of thermic effect of a meal are those of Bradfield (1973), who found no difference in thermic response to isocaloric test meals containing either 40 g protein or no protein in a study with 4 obese women on a weight reducing regime. The determination of the thermic effects of fat, carbohydrate and protein either individually or in combination assumes an adequate intake of vitamins and minerals, (Kleiber, 1945).
Luxusconsumption

The increase in thermic energy produced by certain diets fits in with the concept of luxusconsumption discussed earlier. Miller and Payne (1962) produced luxusconsumption in young pigs on a low protein diet and later Stirling and Stock (1968) were able to maintain the weight of two groups of rats, one of which was consuming 30% more energy than the other group.

Luxusconsumption in man has been demonstrated in diets of varying composition. Both Neumann (1902) and Gulick (1922) were able to maintain their own weight on varying energy intakes. Miller and his group (1967 a and 1967 b) have done a number of experiments on overeating in man in which they found very small weight gains were achieved compared with the extra food consumed. The rate of weight gain fell throughout the overeating period which would suggest that there was an adaptation to the higher food consumption developing. Doyle, et al. (1965), found it necessary to increase the energy intake of young men on a vegetarian diet by 20% in order to maintain their weight. In a successful attempt to produce experimental obesity in man, Sims (1968) found an excess of energy consumption over that predicted was necessary to achieve the 25% gain in weight he desired. He also found a much higher food intake was required to maintain the higher body weight than was required for maintaining their preobese body weight. It was also higher than that required in a group of spontaneously obese subjects. One of the most interesting features of his study was that when the experimentally obese came off the experiment and were no longer encouraged to overfeed they rapidly and spontaneously gravitated towards their habitual weight.
On diets that contained up to a daily ingestion of 6800 Kcal from fat as well as 690 Kcal from carbohydrate and 276 Kcal from protein, normal healthy people (three males and two females) showed only a slight weight gain in the experiment reported by Kasper, Theil and Ehl (1973). The subjects in these experiments when consuming intakes of 300-400 g or higher of fat experienced a marked sensation of heat extending over all the body. There was a difference between the weight response to olive oil and corn oil, the weight gain being less on the corn oil diet. Kasper, et al. concluded that the linoleic acid content which is approximately 45% for corn oil and 7% for olive oil may be an important factor in luxusconsumption.

These results appear to conflict with the work of Forbes, et al. in the 1930's who showed in work with rats that fat improved the economy of food utilisation. Perhaps the most significant difference between the two series of experiments, besides the obvious species difference, was that Kasper was overfeeding adults who had a limited potential for growth whereas Forbes and Swift were working with weanling rats growing at different rates on equicaloric diets of varying nutrient composition.

Drug induced thermogenesis

There are three other common dietary components that have been shown to increase metabolic rate, alcohol (Stock, et al., 1973; Stock and Stewart, 1974; Miller, 1974; Rosenberg and Durmin, 1976) theophylline and caffeine (Miller, 1975). Alcohol was shown to produce only a small increase in oxygen consumption when taken alone but when taken with a meal the thermic effect was considerably larger than for alcohol or the meal alone; 200 mg theophylline caused a 9% increase in metabolic rate and 250 mg caffeine, about the amount found in two cups of strong coffee a 10% increase (Al-Samarrae, et al., 1975).
Figure 4: Thermic responses to meals of different calorie size. Lower curve is for resting subjects, the upper for subjects exercising at the rate of 12 steps of 11 inches/min. All measurements were made 1 hr following the meal.

From Miller, et al., 1967.
Ascorbic acid (2 g) caused a 10% increase and caffeine and ascorbic acid taken together an 18% increase in metabolic rate (Stock and Stewart, 1974; Miller, 1975; Rosenberg and Durnin, 1976).

The size of the thermic effect of meals

Wachholder and Franz (1944); Suzuki (1959) and Stock (1966) found that the thermic effect of meals was related to their energy content as well as the nutrient composition. Miller and Mumford (1967) give two examples in detail of people who showed increased thermic effect with increased meal size. For one subject a 114% increase in meal size caused a 400% increase in the total thermic energy, for the other a 134% increase in meal size caused a 918% increase in total thermic energy. Although the percentage increases are large they represent only a small fraction of ingested energy, 7.4% and 9.6% respectively. These responses were too small to account for the luxusconsumption of their overeating subjects so they estimated 24 hour heat production from 24 hour oxygen consumption measurements made during normal activity and found it correlated fairly well with the energy intake each day. It appeared that activity in some way increased the thermic effect. In a series of experiments on exercising subjects they found that not only was the thermic effect increased by exercise but that the increase in thermic effect with increasing meal size was also greater (Fig. 4).

In a complex series of experiments the results of which are very difficult to interpret, Swindells (1972) claims that she failed to show any significant difference in thermic response between meals that provided $\frac{1}{5}$th, $\frac{1}{3}$rd or $\frac{1}{4}$ of the daily energy intake. In a second experiment, however, she compared the effect of small meals (600 or 700 Kcal) with large meals (900 or 1200 Kcal) and found that the thermic effect after the larger meals
was greater for each subject.

Bray, Whipp and Koyal (1974) found that the thermic effect of a breakfast of varied energy content (350-3000 Kcal) did not appear to be markedly affected by the caloric load.

**Exercise and dietary thermogenesis**

For the last fifteen years there has been considerable interest in the effect of exercise on post prandial thermogenesis. Reinhold (1964) showed a marked increase in oxygen consumption in exercising subjects after a meal that was absent in fasting subjects. On the other hand, Jones, Thomas and Reeves (1965) were unable to detect a statistically significant increase in oxygen cost of a five minute exercise period following a 1000 Kcal meal. Their subjects were in a supine position on a constant work load bicycle ergometer with resistance loads varying between 200 and 350 kg.m. per minute according to the physical fitness of the subject, and a one minute collection of expired air was made 3½ minutes after the beginning of the exercise. The short duration of the task and the one minute estimate of oxygen consumption may not have been adequate to demonstrate the effect. Dagenais, Oriol and McGregor (1966) also failed to show a significant increase of the thermic effect due to exercise, while exercising on a constant load bicycle ergometer at 300 kg.m./min. These workers point out that their results are consistent with those of Jarisch and Liljestrand (1927) who studied the effects of a heavier work load (750 kg.m./min.) following a mixed meal but Bray, et al. (1974) found that work on a bicycle ergometer approximately doubled the thermic effect of breakfast.
As referred to earlier, Miller, et al. (1967) demonstrated that the thermic response to a meal was increased during exercise. The exercise they used was mounting an eleven inch step twelve times per minute for half an hour, one hour after commencement of eating. They found there was no discernable correlation between protein content expressed as g nitrogen or protein calories per cent and the thermic effect of the meal, either in the resting or exercising state. The effect of individual nutrients was further investigated by Goldsmith, et al. (1971) who compared oxygen consumption during exercise before and after test meals of protein (Casilan, Glaxo Research Ltd.), fat (double cream), carbohydrate (glucose), and water. The average increase after protein was 15% (p < 0.001) and after fat 9% (p < 0.02). The increases in oxygen consumption after carbohydrate or water alone were 4% and 2% respectively but were not statistically significant increases.

In 1968 Bradfield, et al. reported a study on the 'priming' effect of activity on the thermic effect of a meal in obese middle-aged women at rest. They showed the mean thermic response to a 750 Kcal test meal of 'Nutraiment' containing 50 g protein, for 5 hours under sedentary conditions was 13% (98 Kcal) over 5 h of the test meal. An activity period (45 minute walk) significantly increased the thermic response to 17% (128 Kcal over 5 h) of the energy value of the meal. In all cases the activity significantly increased the metabolic rate for the five hours measured after the meal, and from the shape of the curves it was estimated that the additional response continued several hours beyond the 5 hours measured.

Swindells (1972) tried to ascertain whether a similar effect would be observed in women of lighter weight. Most of Swindells' subjects were also younger. She found no significant increase in thermic effect due
to exercise either during exercise, as in Miller's experiments, or after
exercise, as in Bradfield's study. Buskirk, Lampetro and Welch (1957)
had previously claimed that moderate exercise (a 4 to 5 mile walk or sled
pull) appeared to produce no additional elevation of dietary thermogenesis.
It is apparent that the influence of exercise on the thermic effect of
meals is variable.

**Nutritional status and dietary thermogenesis**

A well-established feature of the reports on energy balance in
man is the reduction in energy expenditure that is associated with
restricted food intakes and the consequent emaciation (Benedict, 1915;
Dubois, 1936; Keys, et al., 1950; Apfelbaum, et al., 1971). The reduction
in energy losses is achieved by a reduction in the energy cost of activity
(Keys, et al., 1950; Apfelbaum, et al., 1971) as well as a reduction in
basal metabolic rate (Benedict, 1915; Forbes, et al., 1931; Keys, et al.,
1950; Apfelbaum, et al., 1971).

If we suppose that the thermic effect of food is also part of the
system which controls energy balance in man, we might also suppose that
it would be changed in order to conserve energy stores when food intake is
restricted. Of course the thermic effect of food would be reduced anyway,
because not so much food is being consumed, but just as basal metabolic rate
is reduced more than can be explained by the lean body weight loss, so a true
adaptation would mean the thermic effect of food would be reduced more than
can be accounted for by the restricted food intake.
An alternative suggestion is that the thermic effect of food may be the regulator of the upper end of energy balance and the changes in basal metabolic rate and activity the regulator at the lower end.

Very few measurements of the thermic effect of meals in undernourished individuals have been made. Keys, et al. (1950) in their formidable study of 32 young men on restricted food intakes designed to produce a 25% loss of body weight estimated the specific dynamic action of food to be 7.5% of energy intake at the end of their semi-starvation experiment, they did not measure changes in oxygen consumption following a meal. In their review of the pertinent literature they cite the work of Johansson (1908) who found a 75 gm glucose meal failed to increase the metabolic rate of a fasting man and Richardson and Mason (1923) who found an emaciated diabetic patient showed no thermic effect 1 1/2 hours after fat ingestion. The studies which suggest undernutrition does not affect the specific dynamic action, are those of McCann (1920), and Boothby and Bernhardt (1931), and one which indicates specific dynamic action is increased is that of Mason, Hill and Charlton (1927).

More recently Kreiger (1966 & 1969) found that when underweight children were refed large increases in postprandial metabolic rate occurred, and that there was a strong correlation between the rate of growth and the size of the post-prandial thermic effect, faster growth rates being associated with larger thermic effects. He had estimated metabolic rate by measuring insensible loss of weight using a sensitive recording balance, and found that during an initial period when the weight of the infants was kept constant by feeding a maintenance diet, the thermic effect was within the normal range (0-18.5%).
At about the same time Ashworth (1969a & b) and Brooke and Ashworth (1972) published the results of similar studies where the metabolic rate was estimated from direct measurements of oxygen consumption. Contrary to Kreiger they found there was little increase in post-prandial metabolic rate in malnourished infants examined as soon as possible after admission to hospital. However, just as in Krieger's study, when there was rapid growth there was a much larger increase in thermic effect of the meal. After recovery, when the weight gain of the infants had plateaued, the thermic effect was smaller. There was a highly significant correlation (+0.90 p < 0.001), between growth rate expressed as increase in weight as g gain/day and the percentage increase in metabolic rate for 1.75 hours following the meal.

They suggested that the increase in postprandial metabolic rate represented the energy cost of liver protein synthesis, because the postprandial increase was not large enough to account for the total growth observed. A number of figures for the cost of protein synthesis have been suggested (Miller & Payne, 1961; Keilanowski & Kortarbinska, 1969; McCracken, 1973; Close, 1973). Brooke and Ashworth used those of Keilenowski and Kortabinska which are in good agreement with the later estimates of McCracken. The increase in oxygen consumption in the two hours following the meal is less than would account for the two grams of tissue growth which they estimated from daily growth rates occurred in that time.

If the postprandial increase in metabolic rate is due only to liver protein synthesis then their results imply that there is a close correlation between postprandial liver protein synthesis and total overall daily growth rates.
If the thermic effect is due to increased protein synthesis as Ashworth suggests, the absence of a large increase in postprandial metabolic rate in Ashworth's newly admitted malnourished infants does not fit in with the finding of Picou and Taylor Roberts (1969) that in newly admitted malnourished children the rate of total protein synthesis was greater than in children who had recovered from malnutrition.

Patients with anorexia nervosa form another group of people with primary undernutrition where emaciation is due to restricted food intake rather than organic disease. Although there are a number of reports of reduced basal metabolic rate in anorexia nervosa (Du Bois, 1936; Ljunggren, et al., 1961; Crisp, 1965; Russell, 1967) there is only one brief report in the literature of the size of the thermic response to food in this condition. Green, et al. (1975) measured oxygen consumption in a group of 6 anorexia nervosa patients while they were being refed in hospital. They found a 31% increase in oxygen consumption after a 'standard' breakfast. From personal communication it was revealed that this standard breakfast was of 550 Kcal with 18% of the energy from protein, 40% from fat and 42% from carbohydrate, and that the thermic effect was measured by making a 5 minute reading of oxygen consumption at 5 minute intervals commencing 1 hour after the start of breakfast.

It can be seen that there is no conclusive evidence yet that in malnourished individuals the thermic effect of a meal is reduced in order to conserve energy, but there is some evidence in undernourished infants and anorexia nervosa patients once refeeding and rapid weight gain have commenced the postprandial increase in metabolic rate is much greater. At the other end of the scale there is some evidence that in the obese there is a reduced ability to dissipate excess energy.
Early studies include those of Means (1915); Rolly (1921); Plaut (1922); Liebesny (1924), and Wang, Strouse and Saunders (1924). Means (1915) observed the effect of glucose and of meat on the metabolic rate in one case of obesity. He concluded that "the specific dynamic action of protein is as great in this case of obesity as in normal individuals".

The widely quoted paper of Rolly (1921) claims that specific dynamic action is reduced in obesity but can hardly be taken as conclusive evidence because he only studied two patients. One with tuberculosis of the testis was studied before castration when he weighed 56.7 kg and some time after the operation when he weighed 86.1 kg. The other patient was a woman with exophthalmic goitre who, after thyroidectomy and pregnancy, had increased in weight from 57 kg to 89.5 kg. However, in these patients he found the peak response in oxygen consumption following a meal of 1000 g of grated raw meat and one egg yolk occurred approximately 3 hours after the meal. The oxygen consumption at this time increased approximately 50% but after the patients had gained weight the same meal increased oxygen consumption approximately 27% at three hours.

Plaut (1922 and 1923) studied larger numbers of obese individuals (48 altogether) and compared them with normal people and a few people with what she called 'constitutional thinness'. She found the thermic effect of a meal of 200 g of chopped beef, 50 g of fat, and 200 g of bread, one hour after the meal ranged from -5% to +28% in her obese subjects, between +20 and +50% in the normal subjects, and between -40 and +63% in those with constitutional thinness.
Liebesny (1924) reported wide variations in the thermic response to 200 g of roast veal and 100 g of bread in obese persons. He classified them according to their response and his findings are summarised according to his categories in Table 1.

**TABLE 1**

The thermic response to a meal of roast veal and bread in obese persons.

From Liebesny (1924).

<table>
<thead>
<tr>
<th>Number of persons</th>
<th>Peak SDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal BMR with normal SDA</td>
<td>6</td>
</tr>
<tr>
<td>Diminished BMR and normal SDA</td>
<td>24</td>
</tr>
<tr>
<td>Approximately normal BMR &amp; diminished SDA</td>
<td>25</td>
</tr>
<tr>
<td>Low BMR and low SDA</td>
<td>6</td>
</tr>
</tbody>
</table>

The effect of meals of different nutrient composition on specific dynamic action in the obese was reported by Wang, et al. (1924). They compared their eleven obese subjects with 5 underweight and 5 normal subjects. They found a carbohydrate meal (80-106 g of sucrose as lemonade or mint flavoured fondant) caused greater increases in metabolic rate in the thin and normal persons than in the obese. This was also true for the protein meal which varied in size between 32 and 66 g of protein. The fat meal ('sweetened ice cream made from 40% cream, the amount of fat varied from 55-129 g') caused only slight increases in postprandial metabolic rate in all the groups, the average maximum increases were for the obese 5.8%, the thin 10.5%, and the normal 6.8%.
More recently Bradfield (1973), in a study of four obese women on a weight reducing regime found a thermic effect of about 70% when a meal which provided 50% of the maintenance calories was given. This represented 17% of the dose calories. These values for thermic effect are very large and this is mostly because they were calculated as percent increase of the lowest value for basal metabolic rate for each subject. When the thermic effects were compared with the basal metabolic rate value for the day the thermic effect of a meal of 50% of maintenance calories including 40 g of protein was 5% and an isocaloric protein free meal 2%; these values are not significantly different.

Pittet, et al. (1976) measured the thermic effect of 50 g glucose by direct and indirect calorimetry in 11 obese and 10 control female subjects. They found that the metabolic rate measured for 150 minutes after the glucose meal increased 5.2 ± 1.3% in the obese group and 13.0 ± 1.5% in the control group.

The brief report of Green and Miller (1975) also gives a rather low thermic response, 14%, to a standard (unspecified) breakfast in 8 obese women in hospital on a reducing regime of 800 Kcal a day. The thermic response in their anorexia nervosa patients undergoing refeeding was more than twice as large.

Reading the early literature one is struck by the extraordinary nature of the meals. Holly's patients evidently calmly sat down and ate their way through 1 kg of minced beef with one egg yolk; he comments that the patient had a good appetite!
From the literature it would appear that dietary thermogenesis may be an important factor in determining energy balance and particularly important in preventing the tendency to obesity with increased food intakes. If this is so it would be of value to know and understand the biochemical pathways and physiological control mechanisms that enable the wastage of food energy as heat. There have been a number of approaches to these problems; some workers, for example, Stirling and Stock (1968) have looked for biochemical similarities with similar phenomena such as cold induced non-shivering thermogenesis: others, for example, Sims, et al. (1971) have explored the biochemical differences between the obese and people of normal weight. Both of these approaches have yielded useful information.

Biochemical pathways of thermogenesis

The excellent reviews of Gordon (1970) and Stirling and Stock (1973) discuss the biochemical mechanisms that could be responsible for the wastage of food energy. Stirling classified them into three categories:

1. Uncoupling of oxidative phosphorylation
2. Poorly phosphorylating pathways
3. Energetically wasteful cycles.

1. Uncoupling of oxidative phosphorylation

Much of the energy in food is made available by oxidative processes that are coupled to adenosine triphosphate (ATP) synthesis in the mitochondria. For each mole of oxygen consumed usually three moles of ATP are yielded and this is described as a P:O ratio of 3. The P:O ratio
Figure 5: The α-glycerophosphate shuttle.
Numbers in circles refer to "high energy phosphate bonds".

Mitochondrion

Glycerol 1-phosphate \[ \xrightarrow{\text{HEAT}} \] Di-hydroxyacetone phosphate
\[ \xleftarrow{2-P} \]

\[ \xleftarrow{3-P} \] Glycerol 1-phosphate \[ \xrightarrow{\text{HEAT}} \] Di-hydroxyacetone phosphate

NEGLIGIBLE

Cytoplasm
may be reduced with an associated increase in heat production by a number of chemical compounds called uncoupling agents. Free fatty acids are well known uncouplers of endogenous origin and thyroxine in pharmacological doses can also cause uncoupling. Attempts to treat obesity using uncouplers such as dinitrophenol have been largely unsuccessful due to harmful side effects.

2. Poorly phosphorylating pathways

For some substrates there are alternative pathways for oxidation which do not yield so much ATP. P:O ratios of 0.9 have been found for the oxidation of NADH and NADPH and coupling to cytochrome via ascorbate (Kielley and Bronk, 1957; Lehninger, et al., 1954). This is interesting because in 1960 Hoijer found increased ascorbic acid levels in the tissues of cold adapted animals and suggested this could increase thermogenesis in these animals.

Cold adapted animals also show increased activity of mitochondrial α-glycerophosphate dehydrogenase and thus the α-glycerophosphate shuttle (Fig. 5) which generates only two high energy phosphate bonds compared to three which would be the yield via the full electron transport chain.

Succinate is also oxidised through a pathway which has a P:O ratio less than three because the oxidation is flavoprotein linked. An increase in the activity of intramitochondrial α-glycerophosphate dehydrogenase has been produced by dietary means in rat liver (Stirling and Stock, 1968). The rats with the increased enzyme activity were luxusconsuming 30% more food than the control group.
Figure 6: Opportunities for wasteful cycling in the glycolytic chain.
The large arrows indicate possible futile cycles.
A reduced activity of intramitochondrial dehydrogenase has been observed in adipose tissue in some forms of human obesity (Galton, 1966; Bray, 1969). The obese with hypothalamic lesions showed no increase in enzyme activity. The resulting accumulation of α-glycerophosphate would stimulate lipogenesis. In the patients with reduced enzyme activity it could be restored by administration of triiodothyronine.

3. **Energetically wasteful cycles**

The synthesis of triglyceride from glucose and the subsequent degradation to acetyl CoA and oxidation by the Krebs cycle is more energetically wasteful than the direct oxidation of glucose.

Similarly, the esterification of fatty acids with α-glycerophosphate to triglyceride followed by lipolysis and re-esterification of the free fatty acids either within the adipose tissue or at some distant tissue is a very energetically wasteful process. ATP is necessary to activate both the re-cycled free fatty acids and glycerol.

Other opportunities for wasteful cycling occur at four steps in the glycolytic chain. One usually assumes the chain is driven in one direction or the other but if inhibition of enzymes were incomplete at these points cycling could occur (Fig.6).

In fact, any cycle where there is continuous synthesis of macromolecules from simple precursors affords opportunities for wasteful cycling.
The control of each of these processes is complex and outside the scope of this present study but some aspects of control that have been shown to be altered in either cold induced thermogenesis, dietary thermogenesis or obesity are considered below.

Catecholamines have been implicated in dietary thermogenesis since Stirling and Stock (1968) showed that rats that were luxusconsuming had much greater increases in oxygen consumption in response to nor-adrenaline administration. Stirling and Stock had two groups of rats; one group was fed a normal stock diet in amounts sufficient to maintain weight (HP diet), the other group was fed the same stock diet but diluted with fat, sugar and starch (LP diet) such that the concentration of protein was small enough for the rats to maintain weight when fed freely. The animals on the LP diet ate much more food energy than those on the HP diet and yet both groups maintained the same weight. Towards the end of the experiment the response of oxygen consumption to a standard dose of nor-adrenaline (400 µg/kg given subcutaneously) was measured both in fasted and fed animals from the two groups. The increase in oxygen consumption was much greater in the LP diet group; that is those rats that were luxusconsuming showed a greater sensitivity to nor-adrenalin. At the end of the experiment L α-glycerophosphate oxidase levels in liver were measured and found to be higher in the LP diet group. Thus they proposed a model for thermogenesis which is based on the control of intracellular α-glycerophosphate levels, (Stirling and Stock, 1973). When α-glycerophosphate levels are low lipogenesis is likely to be reduced and oxidation of fatty acids will occur. They suggest this is thermogenic because fatty acids have been shown to uncouple oxidative phosphorylation and the steps catalysed by acyl CoA dehydrogenase are FAD linked and so poorly phosphorylating.
This hypothesis is supported by the evidence of Galton and Bray (1967) who found the activity of the α-glycerophosphate shuttle to be reduced in certain types of obesity and Bray, et al. (1969) who found that after restriction of food intake in humans homogenates of adipose tissue had lower activity of both mitochondrial and soluble α-glycerophosphate dehydrogenase. The enhancement of the α-glycerophosphate shuttle by administration of triiodothyronine lends further support to the shuttle pathway as a potential pathway for thermogenesis (Bray, et al., 1969).

Very recently it has been reported that ephedrine, a drug used in the treatment of asthma which stimulates non-adrenaline release, increases oxygen consumption in rodents and man (Massoudi and Miller, 1977 and Evans and Miller, 1977), thus giving further support for the potential role of catecholamines in thermogenesis.

Hormonal control of thermogenesis

The discussion earlier about whether the thermic effect of meals and luxusconsumption are closely related phenomena becomes very important when discussing control mechanisms because any explanation of the meal phenomenon must allow for a control system that is affected by food consumption. There is little evidence that changes in thyroxine or triiodothyronine levels occur postprandially and so it is most unlikely to be part of the control of postprandial thermogenesis. Similarly, it is unlikely that catecholamines are responsible: The thermogenesis associated with catecholamine release may be an important factor in the daily energy balance equation; it may even be greater in prolonged overfeeding (luxusconsumption) as in Stirling's work without being directly involved in the thermic effect of a meal.
Glucagon is another hormone that has been shown to be thermogenic in man (Trisotto, 1971), rat (Davidson, et al., 1960; Miller, et al., 1963), newborn rabbits (Heim, 1966), and dogs (Weiser, 1974). Glucagon promotes glycogenolysis but it is not known to what extent glycogen synthesis is suppressed and thus futile cycling prevented. Glucagon also stimulates gluconeogenesis which has a substantial energy requirement. Theophylline enhances the effect of glucagon on glucose production at low levels of glucagon (Exton, et al., 1971) and this is interesting in the light of Miller's (1975) observation that 200 mg theophylline (about the amount taken in 2 cups of strong tea) caused a 9% increase in oxygen consumption in man. The role of glucagon in lipid metabolism was reviewed by Lefebvre (1972). Theophylline also increases the lipolytic effect of glucagon. As glucagon is a lipolytic hormone, it could increase the wasteful cycling of free fatty acids. Protein synthesis is inhibited by glucagon and protein catabolism and urea synthesis accelerated. This effect on protein turnover may be as a direct effect of the hormone or its messengers or indirectly because of the stimulation of gluconeogenesis. These actions of glucagon may be important in trauma where there is evidence that there is increased glucose turnover and protein catabolism associated with the elevated metabolic rate.

Homeostasis is not achieved by each hormone working in isolation. The actions of glucagon are closely related to those of insulin and there are complex interactions between the two hormones which were reviewed by Samols, Tyler and Marks (1972) and Unger and Lefebvre (1972). As both of these hormones may be secreted in response to eating they may be involved in the control of the metabolic pathways which result in the thermic effect of a meal.
This control may be direct or indirect. Homeostasis for a particular parameter may be achieved at some 'cost' in other ways, for example, blood glucose levels are maintained in short term starvation at the expense of protein catabolism. Changes in energy balance and net lipid synthesis may result as a by product of homeostatic mechanisms for maintaining parameters such as blood glucose rather than by a direct control system which regulates the triglyceride content of the body.
CHAPTER TWO

EXERCISE AND DIETARY THERMOGENESIS IN NORMAL ADULTS
CHAPTER 2

EXERCISE AND DIETARY THERMOGENESIS IN NORMAL ADULTS

First Study
Purpose
Subjects
Experimental design
Procedure
Results
Discussion

Second Study
Purpose
Subjects
Experimental design
Procedure
Results

Third Study
Purpose
Subjects
Experimental design
Procedure
Results

Discussion - the metabolic changes during thermogenesis and exercise

Discussion - the influence of exercise on dietary thermogenesis
First Study

Purpose of the first study

Exercise is one of the few factors of daily life that has been shown under certain conditions to increase the thermic effect of a meal. In previous studies (Jones, et al., 1965; Miller, et al., 1967; Goldsmith, et al., 1971; Swindells, 1972) each group of workers investigated one level of activity and its effect on meals of varying energy or nutrient content. This study was designed to measure the effect of varying exercise levels on the thermic effect of a meal of fixed energy and nutrient content.

The subjects

The subjects were four female university students. Details of their height, weight and age are given in Table 2.

Table 2:

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age Years</th>
<th>Weight kg</th>
<th>Height m</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Female</td>
<td>20</td>
<td>50.9</td>
<td>1.61</td>
</tr>
<tr>
<td>B</td>
<td>Female</td>
<td>20</td>
<td>69.2</td>
<td>1.70</td>
</tr>
<tr>
<td>C</td>
<td>Female</td>
<td>19</td>
<td>60.7</td>
<td>1.60</td>
</tr>
<tr>
<td>D</td>
<td>Female</td>
<td>20</td>
<td>50.0</td>
<td>1.69</td>
</tr>
</tbody>
</table>

Experimental Design

Each subject was studied for one day in five consecutive weeks. All experiments started between midday and 1300 hours so that any variations due to diurnal rhythms in energy expenditure were avoided. In the five week period each subject carried out the following experiments.
Figure 7: Experimental plans for the first study.

Experiment E₀, the subject was at rest throughout.

Experiment E₁, E₂ and E₃, the subject walked on a treadmill before and after a meal.

Experiment E₄, the subject exercised at the moderate rate without a meal in the intervening period.

Experiment E₀

Experiments E₁, E₂ and E₃

Experiment E₄

oxygen consumption measured meal
E0  Resting with a meal
E1  Exercising at 2½ mph on the flat with a meal
E2  Exercising at 2½ mph on a gradient of 8° with a meal
E3  Exercising at 2½ mph on a gradient of 16° with a meal
E4  Exercising at 2½ mph on a gradient of 8° without a meal.

The experiments were carried out on a latin square design so that no effect could be attributed to the subjects becoming trained to the exercise.

Procedure

The subjects refrained from eating on the morning of the day of the test. The experiments followed the general design of Miller, Mumford and Stock (1967) and are illustrated in Figure 7. The exercise used was walking on a treadmill (Andrew Young and Son, Glasgow) at 2½ mph at different gradients. Oxygen consumption was measured for 30 minutes before the meal and 30 minutes after the meal. The meal period was 60 minutes but the food was eaten within the first 15 minutes of the meal period immediately after the first oxygen consumption measurement.

In Experiment E0 the subject was at rest, sitting in a chair for the whole duration of the experiment. In experiment E1 the subject walked for the two 30 minute periods before and after the meal period at 2½ mph on the flat. In Experiment E2 the subject walked at 2½ mph on an incline of 8°. In Experiment E3 the subject walked at 2½ mph on an incline of 16°. Each subject performed the moderate exercise rate, walking at 2½ mph on a gradient of 8°, without eating a meal - experiment E4 - in order to test whether any difference between oxygen consumption of the two exercise periods was in fact due to the meal and not the repetition of the exercise.
Table 3

Test meal used in the first series of experiments.

<table>
<thead>
<tr>
<th>Weight (oz)</th>
<th>Carbohydrate (g)</th>
<th>Protein (g)</th>
<th>Fat (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>3</td>
<td>45</td>
<td>7</td>
</tr>
<tr>
<td>Butter</td>
<td>1</td>
<td>-</td>
<td>Trace</td>
</tr>
<tr>
<td>Cheese</td>
<td>2</td>
<td>-</td>
<td>14</td>
</tr>
<tr>
<td>Corned Beef</td>
<td>2</td>
<td>-</td>
<td>12.5</td>
</tr>
<tr>
<td>Apple</td>
<td>4</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Ribena</td>
<td>2</td>
<td>35</td>
<td>-</td>
</tr>
</tbody>
</table>

Total energy 980 Kcal.
Meal

The food and nutrient composition of the test meal is given in Table 3. The nutrient composition was calculated using food tables (McCance and Widdowson, 1966).

The meal contained approximately 1,000 kilocalories which represented about twice the normal midday intake of the subjects. This energy intake was chosen because it represents nearly half of the Recommended Intake of energy (2,200 Kcal) for sedentary adult women and because Miller and Mumford (1967) had shown that a larger thermic effect is obtained with a meal of high energy content. The same meal was given on each test occasion.

Oxygen consumption measurement

Oxygen consumption was measured using a Kofrani-Michaelis respirometer (Max Plank Institute). This instrument measures the total volume of expired air and takes a continuous sample (either 0.3% or 0.6%) which can subsequently be analysed. For exercise measurements the 0.3% sampling rate was used and for resting measurements the 0.6% sampling rate.

The oxygen content of the sample was analysed with a paramagnetic oxygen analyser (Servomex, Crowborough, Sussex).

Energy expenditure was estimated using Weir's formula (1949). This enables the calculation of energy expenditure without estimation of urinary nitrogen or expired carbon dioxide. Durnin and Passmore (1967) state that the error by not determining CO₂ is ± 0.5% and by not estimating urinary nitrogen ± 1.0%.
Weir's Formula

\[ E \text{ (Kcals/min)} = \frac{4.92}{100} V \left( 20.93 - \text{Exp O}_2\% \right) \]

- \( E \) = energy expenditure, Kcals/minute
- \( V \) = volume of expired air at STP in litres/minute
- \( \text{Exp O}_2\% \) = oxygen content of expired air expressed as a percentage.

Results

The thermic responses to the meal at rest and at different exercise rates are shown in Table 4 and Figure 8. Three subjects A, B and D showed an increase in thermic response with increased energy expenditure, but subject C showed no statistically significant correlation between thermic effect and energy expenditure. The regression and correlation analysis is given in Table 5. The slopes and intercepts for subjects A, B and D are significantly different, \( p < 0.01 \).

The thermic response was not due to mere repetition of the task. There was no statistically significant difference (paired T test) between the first period of moderate exercise and the same exercise repeated one hour later without a meal in the intervening period, Table 6.

A close positive correlation between ventilation rate and energy expenditure was found for each individual and for the whole group, Table 7 and Figure 9.
Table 4

Energy expenditure at rest and at different exercise rates before and after a meal in four young women.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Exercise rate</th>
<th>Energy expenditure before meal Kcal/min</th>
<th>Energy expenditure after meal Kcal/min</th>
<th>Thermic effect expressed as % increase of resting energy expenditure</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>E₀</td>
<td>0.74</td>
<td>0.97</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>2.27</td>
<td>2.69</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>E₂</td>
<td>3.30</td>
<td>3.66</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>E₃</td>
<td>5.38</td>
<td>5.86</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75</td>
<td>0.82</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.30</td>
<td>2.61</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.18</td>
<td>3.66</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.51</td>
<td>6.14</td>
<td>85</td>
</tr>
<tr>
<td>B</td>
<td>E₀</td>
<td>0.83</td>
<td>0.95</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>3.09</td>
<td>3.27</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>E₂</td>
<td>4.03</td>
<td>4.61</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>E₃</td>
<td>5.75</td>
<td>6.50</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.84</td>
<td>0.92</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.02</td>
<td>3.41</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.16</td>
<td>4.80</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.75</td>
<td>6.77</td>
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</tr>
<tr>
<td>C</td>
<td>E₀</td>
<td>1.13</td>
<td>1.20</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>2.96</td>
<td>2.99</td>
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</tr>
<tr>
<td></td>
<td>E₂</td>
<td>4.26</td>
<td>4.06</td>
<td>-20</td>
</tr>
<tr>
<td></td>
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<td>-33</td>
</tr>
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<td></td>
<td></td>
<td>0.92</td>
<td>1.23</td>
<td>34</td>
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<td></td>
<td></td>
<td>2.98</td>
<td>2.87</td>
<td>-11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.78</td>
<td>4.21</td>
<td>-53</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.64</td>
<td>6.75</td>
<td>11</td>
</tr>
<tr>
<td>D</td>
<td>E₀</td>
<td>1.04</td>
<td>1.05</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>2.25</td>
<td>2.40</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>E₂</td>
<td>2.75</td>
<td>3.12</td>
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</tr>
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<td></td>
<td>E₃</td>
<td>5.04</td>
<td>5.74</td>
<td>68</td>
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<td></td>
<td></td>
<td>1.02</td>
<td>1.25</td>
<td>23</td>
</tr>
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<td>2.21</td>
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<td>45</td>
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<td></td>
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<td>2.80</td>
<td>3.14</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.00</td>
<td>5.75</td>
<td>74</td>
</tr>
</tbody>
</table>
Figure 8: The relationship between the thermic effect of a mixed nutrient meal and energy expenditure at different exercise rates in four young women (subjects A, B, C, D). Thermic effect is expressed as % of premeal resting metabolic rate.
Table 5

Correlation between energy expenditure and thermic effect of a meal in four young women.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation Coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>11.17</td>
<td>18.9190</td>
<td>0.73</td>
<td>0.05</td>
</tr>
<tr>
<td>B</td>
<td>19.72</td>
<td>-10.8412</td>
<td>0.93</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>-6.88</td>
<td>17.9963</td>
<td>-0.54</td>
<td>NS</td>
</tr>
<tr>
<td>D</td>
<td>14.70</td>
<td>-3.7604</td>
<td>0.91</td>
<td>0.01</td>
</tr>
</tbody>
</table>
Table 6

Energy expenditure in four young women during two periods of exercise without a meal in between (Experiment Ei).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Energy expenditure in first period Kcal/min</th>
<th>Energy expenditure in second period Kcal/min</th>
<th>% change</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3.21</td>
<td>3.25</td>
<td>+ 1</td>
</tr>
<tr>
<td>B</td>
<td>4.25</td>
<td>4.10</td>
<td>- 4</td>
</tr>
<tr>
<td>C</td>
<td>4.14</td>
<td>4.03</td>
<td>- 3</td>
</tr>
<tr>
<td>D</td>
<td>3.07</td>
<td>2.87</td>
<td>- 7</td>
</tr>
</tbody>
</table>

Paired t test between the first and second exercise periods indicates there is no statistically significant difference between the two exercise periods.
Figure 9: The relationships between ventilation rate and energy expenditure in four young women (subjects A, B, C and D).
Table 7

Correlation between energy expenditure and ventilation rate in four young women.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Intercept</th>
<th>Slope</th>
<th>Correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.92</td>
<td>3.53</td>
<td>0.99</td>
<td>0.001</td>
</tr>
<tr>
<td>B</td>
<td>1.43</td>
<td>3.59</td>
<td>0.99</td>
<td>0.001</td>
</tr>
<tr>
<td>C</td>
<td>2.26</td>
<td>3.51</td>
<td>0.99</td>
<td>0.001</td>
</tr>
<tr>
<td>D</td>
<td>2.93</td>
<td>3.29</td>
<td>0.99</td>
<td>0.001</td>
</tr>
</tbody>
</table>
Discussion

The results suggest that for some individuals (Subjects A, B and D) there is an increase in thermic response with increased physical activity. However, the increase in thermic effect due to exercise is very small compared with the increase in metabolic rate due to the exercise itself. At the $E_3$ exercise level the energy expenditure was at least 500% greater than resting energy expenditure before the meal yet the meal only increased the energy expenditure at $E_3$ exercise level by 9-18%. The increases in metabolic rate due to the meal were also comparatively small for activity levels $E_1$ and $E_2$ which caused increases in energy expenditure by 200% and 400% respectively with thermic effects of between 2% and 21%. It will be noted that the increase in thermic effect with increased energy expenditure is only observed when the values are expressed as percentage increase of resting energy expenditure or as energy expenditure per minute and not when expressed as a percentage increase of energy expenditure during exercise before the meal.

The thermic effects observed in the present series of experiments are of similar magnitude to those observed during the exercise experiments of Goldsmith, et al. (1971); Swindells (1972); Miller, et al. (1967); Jones, et al. (1965) and Bray, et al. (1974).

Subject C did not show any increase in thermic effect with increasing activity; if anything at higher exercise rates the post-prandial energy expenditure was lower. Stock (personal communication) and (Miller and Wise, 1975) have also observed similar results.
Second Study

Purpose of the second study

This series of experiments was planned to extend the number of observations of the effect of exercise on dietary thermogenesis and to observe some metabolic changes associated with the phenomenon.

The subjects

The subjects were four female and two male university students. Details of their height, weight and age are given in Table 8. One other male subject (K) volunteered for one experiment only. Subject J found the blood collection traumatic and did not complete the series of experiments.

Experimental design

Each subject was studied on five days over a period of two weeks. The experiments were carried out in random order so that no effect could be attributed to the subjects becoming trained to the exercise.

Each subject carried out the following experiments:

\[ E_0 \] resting with a meal
\[ E_1 \] exercising at 2½ mph on the flat with a meal
\[ E_2 \] exercising at 2½ mph on a gradient of 8° with a meal
\[ E_3 \] exercising at 2½ mph on a gradient of 16° with a meal
\[ E_4 \] exercising at 2½ mph on a gradient of 8° without a meal.
Table 8

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Age Years</th>
<th>Weight (kg)</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>Female</td>
<td>20</td>
<td>59</td>
<td>1.60</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
<td>20</td>
<td>54</td>
<td>1.60</td>
</tr>
<tr>
<td>G</td>
<td>Female</td>
<td>20</td>
<td>65</td>
<td>1.66</td>
</tr>
<tr>
<td>H</td>
<td>Female</td>
<td>20</td>
<td>63</td>
<td>1.62</td>
</tr>
<tr>
<td>I</td>
<td>Male</td>
<td>20</td>
<td>78</td>
<td>1.81</td>
</tr>
<tr>
<td>J</td>
<td>Male</td>
<td>20</td>
<td>68</td>
<td>1.73</td>
</tr>
<tr>
<td>K</td>
<td>Male</td>
<td>41</td>
<td>76</td>
<td>1.75</td>
</tr>
</tbody>
</table>
Figure 10: Experimental plan for the second study

- Time
- Exercise
- Oxygen consumption measured
- Meal
- Blood sample taken
Procedure

A procedure similar to the first series of experiments was used (Figure 10). Serial blood samples were also taken. A polythene cannula was inserted in the anterior cubital vein. Blood was withdrawn from the cannula either with a syringe or by removing the rubber cap and allowing the blood to drip into the collecting tube. The latter method was found to be more convenient, particularly at the high exercise rates because the blood flowed so readily. 10 ml samples were collected into a tube with a paper clip, rocked gently for five minutes on a rotary shaker, then centrifuged. The serum was removed and kept deep frozen at -20°C, until the end of the series of experiments. One ml of whole blood from each collection was placed in a fluoride coated pot for blood glucose estimation. The cannula was kept patent with sodium citrate (Evans Laboratories).

Blood sample 1 was taken when the cannula was inserted.

Blood sample 2 was taken 5 minutes before the first exercise period.

Blood sample 3 was taken 5 minutes after the beginning of the first exercise period.

Blood sample 4 was taken 5 minutes before the end of the first exercise period.

Blood sample 5 was taken 3 minutes after the end of the first exercise period.

Blood sample 6 was taken 30 minutes after the end of the first exercise period.

Blood sample 7 was taken 5 minutes before the second exercise period.

Blood sample 8 was taken 5 minutes after the start of the second exercise period.

Blood sample 9 was taken 5 minutes before the end of the second exercise period.

Blood sample 10 was taken 3 minutes after the end of the second exercise period.
Table 9

Test meal for the second series of exercise experiments.

<table>
<thead>
<tr>
<th></th>
<th>Weight</th>
<th>Carbohydrate</th>
<th>Protein</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bread</td>
<td>120</td>
<td>63.2</td>
<td>9.4</td>
<td>1.7</td>
</tr>
<tr>
<td>Butter</td>
<td>30</td>
<td>-</td>
<td>0.2</td>
<td>26.0</td>
</tr>
<tr>
<td>Cheese</td>
<td>100</td>
<td>Trace</td>
<td>23.0</td>
<td>30.1</td>
</tr>
<tr>
<td>Apple</td>
<td>120</td>
<td>14.0</td>
<td>0.4</td>
<td>Trace</td>
</tr>
<tr>
<td>Milk</td>
<td>30</td>
<td>1.4</td>
<td>1.0</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Total energy 877 Kcals

The milk was taken in a cup of weak instant coffee.
The meal

The food and nutrient composition of the meal chosen by the subjects is shown in Table 9.

The meal differed from that in the first series because some of the volunteers did not like corned beef and it was difficult to get other volunteers for such long experiments.

Determination of serum glycerol

Serum glycerol levels were determined by the method of Laurell and Tibbling (1966). Glycerol is converted to glycerol-1-phosphate by glycerokinase and adenosine triphosphate. Under the influence of glycerolphosphate dehydrogenase and nicotinamide adenine dinucleotide (NAD), dihydroxyacetone phosphate and reduced NAD are formed in proportion to the glycerol content of the serum. The formation of NADH is determined fluorometrically.

Determination of serum non-esterified fatty acids

A kit (Boehringer, Mannheim) was used for the determination of serum non-esterified fatty acid levels. The method is based on that of Howorth, Gibbard and Marks (1966).

Determination of blood glucose

Glucose estimations on samples of whole blood were carried out by the glucose oxidase method (Huggett and Dixon, 1957) using the Boehringer kit (Boehringer, Mannheim).
Results

The thermic responses to the meals at rest and at various exercise levels are shown in Tables 10a and 10b.

Contrary to earlier findings there was no significant correlation between energy expenditure and thermic effect of any of the subjects in this series of experiments. The relationships between energy expenditure and thermic effect are shown in Figure 11 and Table 11. As in the first series of experiments there was no statistically significant difference between energy expenditure when the physical activity was repeated one hour later without a meal in the intervening period (Table 12).

Effect of the meal on blood glucose concentration (Figure 12)

In each of the experiments (E0, E1, E2 and E3) where a meal was taken the blood glucose values were statistically significantly higher 20 minutes and 50 minutes after the start of the meal (p < 0.05). The highest mean value was at 50 minutes but some subjects had their peak blood glucose level at 25 minutes after the meal.

Effect of exercise on blood glucose concentrations (Figure 12)

Blood glucose levels did not change significantly during exercise before the meal.

Blood glucose levels decreased during the exercise period following the meal but blood glucose was also falling at this time in the resting experiment. However, the fall in blood glucose from the peak value was greater (p < 0.05) in the exercise experiments than in the resting experiment. There was no fall in blood glucose during the second period of exercise when no meal had been given in the intervening period.
Table 10a and 10b

Energy expenditure at rest and at different exercise rates
before and after a meal in four women and three men.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Exercise rate</th>
<th>Energy expenditure before meal Kcal/min</th>
<th>Energy expenditure after meal Kcal/min</th>
<th>Thermic effect expressed as % increase of resting energy expenditure</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>E₀</td>
<td>1.09</td>
<td>1.39</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>2.83</td>
<td>3.09</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>E₂</td>
<td>4.20</td>
<td>4.02</td>
<td>-25</td>
</tr>
<tr>
<td></td>
<td>E₃</td>
<td>5.42</td>
<td>5.69</td>
<td>29</td>
</tr>
<tr>
<td>F</td>
<td>E₀</td>
<td>0.94</td>
<td>1.28</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>3.32</td>
<td>3.52</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>E₂</td>
<td>4.08</td>
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<td>G</td>
<td>E₀</td>
<td>0.96</td>
<td>1.36</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>3.49</td>
<td>3.28</td>
<td>-32</td>
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<td>E₂</td>
<td>3.72</td>
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<td>16</td>
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<td>E₃</td>
<td>5.06</td>
<td>5.35</td>
<td>30</td>
</tr>
<tr>
<td>H</td>
<td>E₀</td>
<td>0.92</td>
<td>1.12</td>
<td>22</td>
</tr>
<tr>
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<td>E₁</td>
<td>3.15</td>
<td>3.50</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>E₂</td>
<td>4.21</td>
<td>4.53</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>E₃</td>
<td>6.71</td>
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</tr>
<tr>
<td>Subject</td>
<td>Exercise rate</td>
<td>Energy expenditure before meal Kcal/min</td>
<td>Energy expenditure after meal Kcal/min</td>
<td>Thermic effect expressed as % increase of resting energy expenditure</td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
<td>----------------------------------------</td>
<td>---------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>I</td>
<td>E₀</td>
<td>1.24</td>
<td>1.62</td>
<td>27.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.99</td>
<td>1.70</td>
<td>-14.6</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
<td>3.13</td>
<td>3.55</td>
<td>49.4</td>
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<td>3.42</td>
<td>3.84</td>
<td>49.4</td>
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<td>E₂</td>
<td>4.55</td>
<td>5.03</td>
<td>35.3</td>
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<td></td>
<td></td>
<td>4.82</td>
<td>5.29</td>
<td>34.6</td>
</tr>
<tr>
<td></td>
<td>E₃</td>
<td>6.27</td>
<td>6.63</td>
<td>26.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6.56</td>
<td>6.93</td>
<td>27.2</td>
</tr>
<tr>
<td>J</td>
<td>E₀</td>
<td>1.02</td>
<td>1.74</td>
<td>70.6</td>
</tr>
<tr>
<td></td>
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<td>1.74</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>E₁</td>
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<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>E₂</td>
<td>4.20</td>
<td>4.57</td>
<td>33.9</td>
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<td>52.3</td>
</tr>
<tr>
<td></td>
<td>E₃</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>E₃</td>
<td>6.40</td>
<td>6.46</td>
<td>7.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.43</td>
<td>7.25</td>
<td>-20.9</td>
</tr>
</tbody>
</table>
Figure 11: The relationship between the thermic effect of a mixed nutrient meal and energy expenditure at different exercise rates in four young women (subjects E, F, G and H). Thermic effect is expressed as % of premeal resting metabolic rate.
Table 11

Correlation between energy expenditure and thermic effect in four young women (subjects E.F.G.H.) and one young man (subject I).

<table>
<thead>
<tr>
<th>Subject</th>
<th>Slope</th>
<th>Intercept</th>
<th>Correlation coefficient</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>-5.4</td>
<td>36.20</td>
<td>-0.45</td>
<td>NS</td>
</tr>
<tr>
<td>F</td>
<td>2.3</td>
<td>28.80</td>
<td>0.20</td>
<td>NS</td>
</tr>
<tr>
<td>G</td>
<td>-11.1</td>
<td>38.33</td>
<td>-0.37</td>
<td>NS</td>
</tr>
<tr>
<td>H</td>
<td>-3.6</td>
<td>34.09</td>
<td>-0.64</td>
<td>NS</td>
</tr>
<tr>
<td>I</td>
<td>2.3</td>
<td>20.21</td>
<td>0.22</td>
<td>NS</td>
</tr>
</tbody>
</table>
Table 12

Energy expenditure in five subjects during two periods of exercise without a meal in between (Experiment E4)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Energy expenditure in first period Kcal/min</th>
<th>Energy expenditure in second period Kcal/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>E</td>
<td>4.04</td>
<td>4.09</td>
</tr>
<tr>
<td></td>
<td>4.35</td>
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<td>F</td>
<td>4.24</td>
<td>4.18</td>
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<tr>
<td></td>
<td>4.28</td>
<td>4.19</td>
</tr>
<tr>
<td>G</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>4.58</td>
<td>4.50</td>
</tr>
<tr>
<td></td>
<td>4.85</td>
<td>4.96</td>
</tr>
<tr>
<td>I</td>
<td>5.04</td>
<td>5.07</td>
</tr>
<tr>
<td></td>
<td>5.20</td>
<td>5.15</td>
</tr>
<tr>
<td>J</td>
<td>4.19</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4.15</td>
<td>4.49</td>
</tr>
</tbody>
</table>

Paired t test between the first and second exercise periods indicates there is no statistically significant difference between the two exercise periods.
Blood Glucose mean ± SE

Figure 12: (see Legend on separate sheet)
Blood glucose levels in six young adults (four women and two men) values indicate mean ± S.E. Experiment E_0 the subjects rested before and after a mixed nutrient meal. Experiment E_1, E_2, E_3 subjects exercised for 1 hour before and after the meal on gradients of 0°, 8° and 16° respectively. Experiment E_4 the subjects exercised on a gradient of 8° without a meal.
Blood glucose levels increased after the end of the second exercise period at every exercise level following a meal but not when no meal had been given.

**Effect of the meal on serum non-esterified fatty acid concentrations**
(Figure 13)

There was no change in serum non-esterified fatty acids before or after the meal when the subjects were at rest.

Post-prandial serum non-esterified fatty acid levels were consistently lower following the meal at exercise level E_2 when compared with exercise at the same rate without a meal.

**Effect of exercise on serum non-esterified fatty acid concentrations**
(Figure 13)

Non-esterified fatty acid levels increased during the first exercise period (p < 0.05). At the highest exercise rate there was a marked increase just after exercise had ceased. The NEFA levels gradually returned to fasting values following the meal after the first exercise period. There was no increase in NEFA levels during the second exercise period when a meal had been taken but there was a small increase just after the exercise had finished (p < 0.05).

**Effect of the meal on serum glycerol concentrations**
(Figure 14)

Serum glycerol levels remained constant both before and after the meal when the subjects were at rest.
Figure 13: (see Legend on separate sheet)
Figure 13 Legend:

Serum non-esterified fatty acid levels in six young adults (four women two men) values indicate mean ± S.E. Experiment E₀ the subjects rested before and after a mixed nutrient meal. Experiments E₁, E₂ and E₃ the subjects exercised for ½ hour before and after the meal on gradients of 0°, 8° and 16° respectively. Experiment E₄ the subjects exercised on a gradient of 8° without a meal.
Serum Glycerol mean ± SE

Figure 14: Serum glycerol levels in six young adults (four women two men) values indicate mean ± S.E. Experiment E₀ the subjects rested before and after a mixed nutrient meal. Experiments E₁, E₂ and E₃ the subjects exercised for ½ hour on gradients of 0°, 8° and 16° respectively. Experiment E₄ the subjects exercised on a gradient of 8° without a meal.
Post-prandial serum glycerol concentrations were lower following the meal at exercise rate $E_2$ when compared with exercise at the same rate without a meal. Glycerol levels fell rapidly following the meal at the end of the first exercise period.

**Effect of exercise on serum glycerol concentrations** (Figure 14)

Serum glycerol concentrations generally increased during exercise and just after exercise both before and after the meal. At the highest energy expenditure the increase in glycerol was greatest. The increase in glycerol levels during the second exercise period tended to be smaller than the increase during the first exercise period.

**Discussion**

These results are discussed together with those of the next series of experiments on page 83.

**Third Study**

**Purpose of the third study**

The experimental design of Miller, *et al.* (1967) used in the previous experiments does not exclude the possibility that exercise before the meal might influence the thermic effect during exercise following the meal. The third study was designed to test whether exercise before the meal influenced the thermic effect of exercise following the meal.

**The subjects**

The subjects were four male university students. Details of their height, weight and age are given in Table 13.
<table>
<thead>
<tr>
<th>Subject</th>
<th>Height m</th>
<th>Weight kg</th>
<th>Age Years</th>
</tr>
</thead>
<tbody>
<tr>
<td>K</td>
<td>1.73</td>
<td>69.9</td>
<td>21</td>
</tr>
<tr>
<td>L</td>
<td>1.86</td>
<td>80.4</td>
<td>19</td>
</tr>
<tr>
<td>M</td>
<td>1.76</td>
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</tr>
<tr>
<td>N</td>
<td>1.82</td>
<td>66.4</td>
<td>18</td>
</tr>
</tbody>
</table>
Experimental design

Each subject carried out the following experiments in random order.

Experiment A the subjects rested before and after the meal
Experiment B the subjects rested before the meal and exercised after the meal
Experiment C the subjects exercised before and after the meal.

The exercise was walking on a treadmill at 3 mph on an incline of 8°.

Procedure

The subjects refrained from eating for 12 hours prior to each experiment. The procedure was similar to the second study, except that the meal period was shortened to 30 minutes (Figure 15). Serial blood samples were taken by a similar procedure to that used in the second study.

Blood sample 1 - on cannulation.
Blood sample 2 - five minutes before the first 30 minutes period of exercise or rest.
Blood sample 3 - five minutes after the start of the first 30 minute period of exercise or rest.
Blood sample 4 - five minutes before the end of the first 30 minute period of exercise or rest.
Blood sample 5 - three minutes after the end of the first 30 minute period of exercise or rest.
Blood sample 6 - five minutes before the start of the second 30 minute period of exercise or rest.
Figure 15: Experimental plans for the third study.

**Experiment A**

- 1 - Rest
- 2
- 3 - Rest
- 4
- 5

Time (min): 0, 30, 60, 90

**Experiment B**

- 1 - Rest
- 2
- 3 - Exercise
- 4
- 5

Time (min): 0, 30, 60, 90

**Experiment C**

- 1 - Exercise
- 2
- 3 - Exercise
- 4
- 5

Time (min): 0, 30, 60, 90

Δ denotes period during which oxygen consumption was measured

Δ blood sample taken
Blood sample 7 - five minutes after the start of the second 30 minute period of exercise or rest.

Blood sample 8 - five minutes before the end of the second 30 minute period of exercise or rest.

Blood sample 9 - three minutes after the end of the second 30 minute period of exercise or rest.

Blood sample 10 - ten minutes after the end of the second 30 minute period of exercise or rest.

The meal

The meal was the same as that used in the second study.

Results

The energy expenditure during each experiment is shown in Table 14 and Figure 16.

Experiment A

Comparisons between energy expenditure in periods 1 and 4, and periods 2 and 5 were made using the paired t test. There was a significant increase in metabolic rate following the meal, p < 0.001.

Experiment C

Comparisons between energy expenditure in periods 2 and 5, and periods 3 and 6 were made with the paired t test. There was a significant elevation of metabolic rate following the meal during the second exercise period, p < 0.05 (one tailed test).

Comparison was made between the thermic effect of the meal at rest (Experiment A) and during exercise (Experiment C) (Table 15). In each case the thermic effect was estimated as the difference between the two
Figure 16: Energy expenditure before and after a meal in four young men. Experiment A - no exercise. Experiment B - exercise after the meal. Experiment C - exercise before and after a meal. The numbers denote the periods during which oxygen consumption was measured.
Energy expenditure (Kcal/min) before and after a meal in four young men. The times to which the periods correspond are given in Figures 15 and 16.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Experiment A</th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>O</th>
<th>Mean</th>
<th>SE</th>
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<tr>
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<td>1.20</td>
<td>1.13</td>
<td>1.26</td>
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</tr>
<tr>
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<td></td>
<td>1.29</td>
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<td>1.21</td>
<td>1.14</td>
<td>1.28</td>
<td>0.08</td>
</tr>
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<td>0.05</td>
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<tr>
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<tr>
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<td>1.21</td>
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<td>1.19</td>
<td>1.22</td>
<td>1.25</td>
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Table 15

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</tr>
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<td>O</td>
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<td>9.7</td>
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</table>
corresponding periods before and after the meal expressed as a percentage of the premeal resting metabolic rate. Exercise did not significantly increase the thermic effect.

The results from Experiments B and C were compared to determine whether the period of exercise preceding the meal had any effect on the energy expenditure after the meal. The metabolic rate during exercise following the meal was not significantly altered by a period of exercise before the meal.

**Effect of a meal and exercise on blood glucose concentrations** (Figure 17)

Blood glucose levels remained constant before the meal, both when the subjects were at rest and when they exercised before the meal. Following the meal the glucose levels increased rapidly to peak values approximately one hour after the meal. The post-prandial increase in glucose was slightly lower when subjects exercised following the meal but shortly after exercise ceased there was a sharp increase in blood glucose levels \( p < 0.05 \).

**Effect of a meal and exercise on serum insulin concentrations** (Figure 18)

The serum insulin curve followed the glucose curve closely when subjects rested both before and after the meal (Experiment A).

During exercise following the meal insulin levels were low in relation to the blood glucose levels at that time, but within 3 minutes after the end of exercise there was a rapid increase which continued until 10 minutes after the end of exercise. The increase in insulin after the exercise preceded the increase in blood glucose.
Figure 17: Blood glucose levels in four young men before and after a mixed nutrient meal.
Figure 18: Serum insulin levels in four young men before and after a mixed nutrient meal.
Figure 19: Serum glycerol levels in four young men before and after a mixed nutrient meal.
Figure 20: Serum non-esterified fatty acid levels in four young men before and after a mixed nutrient meal.
Changes in serum glycerol and non-esterified fatty acid levels associated with a meal and exercise (Figures 19 and 20)

When subjects rested both before and after the meal serum glycerol decreased slightly to a nadir approximately 50 minutes after the meal, and NEFA levels gradually decreased to the lowest value 65 minutes after the meal.

Exercise before and after the meal increased serum glycerol levels. Non-esterified fatty acid levels increased during exercise before the meal with a sharp increase just after this exercise period ceased.

In Experiment B during exercise following the meal, non-esterified fatty acid levels at first fell more sharply than when subjects were at rest, then increased slightly. However, in Experiment C when subjects exercised before and after the meal, the levels after the meal fell sharply then continued to fall during the exercise period but then increased when exercise ceased.

Discussion - the metabolic changes during dietary thermogenesis and exercise.

Two spheres of interest have extended the study of energy metabolism during exercise and following the intake of carbohydrate; firstly, the study of the metabolism and requirement of nutrients for athletic performance, and secondly, the benefit of exercise in diabetes mellitus. Increased energy demands during exercise are met by increased oxidation of a variety of substrates of which the most important are glucose and non-esterified fatty acids. The endogenous energy reserves of glycogen and lipids stored in the muscle are limited and therefore the fuel supply to the muscle cell from sources outside the muscle must be substantially increased during exercise. Non-esterified fatty acids are mobilised through
lipolysis occurring mainly in the adipocytes, and the liver is the
dominant and probably the only source of the increased amounts of glucose
utilised during exercise (Wahren, et al., 1975). The quantitative
contribution of glucose and non-esterified fatty acids varies with the
severity of the exercise, the duration of the activity, the previous diet,
and whether the individual had fasted or fed just before the exercise. The
direct measurement of organ or tissue blood flow and arteriovenous
differences (Wahren, et al., 1975) and the use of isotopic tracers (Issekutz,
et al., 1975; Reichard, et al., 1961) have facilitated a more detailed
understanding of these changing patterns of substrate utilisation during
exercise.

Although there are reports of the effects of exercise following
oral administration of glucose (Davidson and Albrink, 1967; Pruett and
Oseid, 1970; Reinheimer, et al., 1968), starch hydrolysate (Orava, et al.,
1974) and sucrose (Benade, et al., 1973), little is known of the effects of
exercise following meals of mixed nutrient composition. The present study,
although not designed specifically to investigate exercise metabolism;
allows some observations to be made.

Both series of experiments demonstrate the constancy of blood
glucose during short-term exercise in fasting man. Although non-esterified
fatty acids are the main fuel for exercise in fasting man (Wahren, et al.,
1975), glucose still makes an important contribution particularly at high
work loads and in exercise of short duration. Wahren, et al. (1975) found
that between 28% and 37% of the total substrate consumption by exercising
leg muscle in man may be accounted for by blood glucose in a 40 minute
exercise period. They also observed splanchnic glucose output rose
progressively during the exercise period and that most of this glucose
output was derived from glycogenolysis. Vranic and Wrenshall (1969) found in exercising dogs that the increased rate of glucose production by the liver and the increased rate of glucose utilisation by the muscle are synchronous and proportional. In later investigations Vranic, et al. (1975) concluded that insulin is the important regulatory hormone during such exercise, the decrease in portal insulin concentrations facilitating glycogenolysis and gluconeogenesis in the liver. The decrease in portal insulin supply during exercise is brought about partly by the reduced blood flow, and partly by a more rapid removal of insulin by muscle cells (Vranic, et al., 1975; Garratt, et al., 1972).

It is unlikely that changes in glucagon levels are responsible for the increase in glycogenolysis during moderate exercise of short duration in fasting man. Blood glucagon concentrations increase during strenuous exercise in dogs (Böttger, et al., 1972; Vranic, et al., 1975) and rats (Luyckx and Lefebvre, 1974), but in man glucagon concentrations do not change (Sutton, et al., 1969) or increase only moderately (Böttger, et al., 1972; Felig, et al., 1972). The duration of activity appears to be an important factor in determining the glucagon response. Galbo, et al. (1975) observed a three-fold increase in glucagon levels during prolonged exercise in man but no statistically significant increase during runs of ten minutes duration at varying work loads.

The increased activity of the sympathetic nervous system associated with exercise results in increased lipolysis in adipose tissue. In the present studies exercise before the meal was usually associated with increases in non-esterified fatty acid levels during exercise and a sharp increase just after exercise ceased. This sharp increase was probably due to the sudden decrease in uptake by muscle. Following the meal exercise did not increase lipolysis to such an extent. The results of the third
study show that insulin levels were higher during exercise following the meal and thus probably suppressed lipolysis and promoted re-esterification. Plasma glycerol levels also reflected the extent of lipolysis and increased more during exercise before the meal. The major site of glycerol uptake is the liver and glycerol is a useful substrate for gluconeogenesis particularly in prolonged exercise in fasting man as it represents a genuine new synthesis of carbohydrate from a source stored in the adipose tissue unlike lactate from the working muscle (Shaw, et al., 1975).

The present studies indicate that during exercise following a meal (including carbohydrate) the energy substrates are different from those during exercise in fasting man. In the second study the fall in glucose was more rapid during exercise than when subjects were at rest and the small increase immediately after exercise ceased probably reflects the sudden decrease in uptake by muscle. In the third study the exercise period was 30 minutes after the meal and earlier than in the second study. In this case glucose levels did not increase as much as when the meal was followed by rest, but there was an increase in glucose levels between 3 and 10 minutes after the end of exercise. In the second study the increase following exercise occurred within 3 minutes. Reinheimer, et al. (1968) also found a rapid increase in glucose levels following exercise during a glucose tolerance test, and that those higher levels were maintained 1½ hours after the end of exercise.

Discussion - the influence of exercise on dietary thermogenesis.

It is apparent from the present studies and a rather small number of similar studies that dietary thermogenesis is very variable during exercise. Questions that may be raised are:

1. Is energy expenditure greater during exercise following a
meal than before a meal?

2. Does exercise potentiate the thermic effect of a meal?

3. Which factors influence the exercise potentiation of dietary thermogenesis?

4. Is dietary thermogenesis part of a mechanism whereby energy output is controlled?

5. What are the mechanisms, if any?

Several groups of workers have found that the energy cost of exercise is increased following a meal (Miller, et al., 1967; Goldsmith, et al., 1971; Bradfield, et al., 1968; Dagenais, et al., 1966; Bray, et al., 1974). Others have found that the cost of exercise is unaffected by a meal (Swindells, 1972; Jones, et al., 1965) and yet others have found the energy cost of exercise to be reduced following a meal (Miller and Wise, 1975; Tuttle, et al., 1952). It will be noted that there are more records of an increased energy cost of postprandial exercise. This does not necessarily mean the weight of evidence is in favour of it, just that the weight of published evidence suggests that the cost of exercise after a meal is increased. There is often reticence to publish negative or inconclusive findings. However, in the present studies, in 13 out of 15 subjects the cost of exercise was greater after a meal than before and most of these subjects were tested three times. This study thus supports the balance of evidence in the literature, but also confirms that this is not true for all individuals.

It has been suggested not only that the thermic effect of a meal can be shown in exercising man but that exercise potentiates the thermic effect of food (Miller, et al., 1967; Bray, et al., 1974). It is more difficult to evaluate the evidence for this; in some studies only the thermic effect during exercise was measured (Goldsmith, et al., 1971);
Table 16

<table>
<thead>
<tr>
<th>Previous day's diet</th>
<th>4 mj</th>
<th>10 mj</th>
<th>17 mj</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost of exercise before breakfast (Kilojoules/minute)</td>
<td>2.8</td>
<td>2.4</td>
<td>2.6</td>
</tr>
<tr>
<td>Cost of exercise after breakfast (Kilojoules/minute)</td>
<td>1.8</td>
<td>2.3</td>
<td>3.0</td>
</tr>
</tbody>
</table>

From Miller and Wise (1975)
in others the design of the experiment or the statistical treatment of the results does not allow firm conclusions to be drawn (Dagenais, et al., 1966; Jones, et al., 1965). Several factors including meal size, previous diet and level of activity, appear to influence the exercise potentiation of dietary induced thermogenesis. Both Miller, et al. (1967) and Bray, et al. (1974) found no exercise potentiation when the meal was less than 1,000 kilocalories, but when the meal exceeded 1,000 kilocalories the thermic effect of the meal was approximately doubled. However, Bradfield, et al. (1968) was able to show exercise potentiation of thermogenesis after a 750 kilocalorie meal in obese women. Meal size has been claimed to influence the thermic effect of food at rest as well (Miller, et al., 1967), but Bray, et al. (1974) and Swindells (1972), did not find the thermic effect of food at rest was influenced by meal size. When the thermic effect of a meal at rest is shown to be so variable it is even more difficult to be sure that exercise potentiates the thermic effect.

In Miller's early work the subjects were overeating and he proposed that the excess calories were wasted during activity in the postprandial state (Miller, et al., 1967). Bray, et al. (1974), who also overfed volunteers did not confirm Miller's findings but observed that the magnitude of the potentiation of thermic effect by exercise had not changed after 28 days of overeating. Miller and Wise (1975) later showed that the food intake of the previous day influenced the cost of exercise following a meal. Their results are summarised in Table 16.

It will be noted that only when the food intake of the previous day was high (17 mj) did the cost of exercise increase after a 3.9 mj breakfast. In fact, when the food intake had been low or moderate the postprandial cost of exercise was lower. They concluded that these results suggest that there is some mechanism influencing energy output and
Figure 21: The effect of breakfast on energy expenditure during steady-state exercise at the work rates indicated. Figures in brackets indicate the time (minutes) after the meal which corresponds with each work rate.

(adapted from Bray, et al., 1974).
therefore tending to regulate energy balance.

Apart from the present studies the influence of level of activity on the thermic effect of a meal has only been investigated by Bray, et al. (1974). Unfortunately, the design of his experiment was not really suitable. The subjects performed an incremental work test before and after a meal. He then compared the linear relationship between oxygen consumption and work rate before and after the meal. The results are presented in Figure 21. Each work rate corresponds to a different time postprandially and oxygen consumption following a meal would be expected to increase at these times independently of the different work rates. Thus, it is not possible to separate the influence of work rate on thermic effect from the gradual increase in oxygen consumption expected at that time postprandially. Thus, his studies indicate that exercise potentiates thermogenesis but not that this potentiation increases with increase in work rates.

The findings of the present studies are also inconclusive as only three of the 9 subjects investigated showed a statistically significant increase in thermic effect with increased level of activity.

If exercise does potentiate thermogenesis one might reasonably predict that there would be a greater exercise potentiation at high work rates. The majority of subjects showed no such exercise potentiation of thermogenesis in the present study. It has been suggested that exercise potentiation of the thermic effect of meals is part of a regulatory mechanism for energy balance (Miller, 1975). This is unlikely. The extra energy
expended in postprandial exercise is very small compared to the cost of exercise itself. Modifications in energy balance could be made much more readily by changing either the duration of activity, or the energy cost of activities before as well as after meals. It is also difficult to imagine a regulatory mechanism which is dependent on the essentially voluntary and variable pursuit of physical activity. Energy expenditure is greater during exercise following a meal than before a meal, but the thermic effect of a meal is not potentiated by exercise.
CHAPTER THREE

WEIGHT GAIN, THERMIC EFFECT OF GLUCOSE AND RESTING METABOLIC RATE DURING RECOVERY FROM ANOREXIA NERVOSA
CHAPTER 3

WEIGHT GAIN THERMIC EFFECT OF GLUCOSE AND RESTING METABOLIC RATE
DURING RECOVERY FROM ANOREXIA NERVOSA

Purpose

Subjects

Treatment for anorexia nervosa patients

Experimental procedure

Results

- weight gain of anorexia nervosa patients during rehabilitation
- resting metabolic rate in anorexia nervosa patients
- thermic response to glucose in anorexia nervosa patients

Discussion

Conclusion
WEIGHT GAIN, THERMIC EFFECT OF GLUCOSE AND RESTING METABOLIC RATE DURING RECOVERY FROM ANOREXIA NERVOSA

Purpose of the study

Anorexia nervosa is a condition in which severe voluntary restriction of carbohydrate intake, and in some cases restriction of consumption of all foods, results in weight loss. Primary or secondary amenorrhea is always present as are a number of physical and psychological stigmata (Crisp, 1965; Russell, 1967). Frequently, the anorectic is previously obese, and, in them, it is clinically expedient to be able to predict, and control, the rate of weight gain in order to give the patient confidence that she will not, once more, become overweight. Kanis, et al. (1974), after summarizing the treatments that have been used said, 'Rational therapy depends on information which at present is still not available'. The purpose of this study was to investigate dietary thermogenesis in relation to the rate of weight gain, especially in the light of reports (Kreiger, 1966; Kreiger and Whitten, 1969; Ashworth, 1969; Brooke and Ashworth, 1972) that there is an increase in the thermic effect of a meal in infants recovering from malnutrition and that when the recovery growth is most rapid the post-prandial metabolic rate is greatest. Hippocrates (460-370 B.C.) noted the high energy requirement for growth and recorded his observations for posterity in Apherism 14, 'Growing bodies have the most heat, they therefore require most food....'. More recently Millward, et al. (1977) reviewed the energy cost of growth in man and certain other species.
The Subjects

Fifteen female patients with anorexia nervosa being treated as in-patients at St. George's Hospital, Department of Psychiatry were chosen for the study. The population was heterogeneous in respect of present weight disorder (Table 17), thus not all of the subjects were severely emaciated before the start of treatment, although all still had anorexia nervosa and had, at some recent time, been severely emaciated. On admission patients 168, 192, 206 and 214 all weighed within the average weight for their height and age but displayed bizarre eating habits in addition to the other clinical features of anorexia nervosa, including secondary amenorrhoea. Eight patients had been at least 20% above the average weight for their height and age at some time prior to the onset of anorexia nervosa. These are referred to as the previously obese (PO) group. Seven patients had no history of previous obesity and are referred to as the previously non-obese (NO) group.

The patients were compared with a group of six female students of similar age and height and who were very close to their target weight for height and age (Table 18). The weights of these subjects were stable and none had suffered from a weight problem or had been over-interested in food or dieting.

Individual details of the height, weight and target weight of the anorectics and their controls are given in Table 18.

The treatment for anorexia nervosa patients

Apart from psychotherapy, the treatment consisted of re-feeding and complete bedrest until a target weight was reached. The target weight for each patient was the average weight of the population matched for their
Weight deficit and extent of re-feeding of anorexia nervosa patients on test occasions.

<table>
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<th>TEST 3</th>
<th>TEST 4</th>
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</thead>
<tbody>
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<td></td>
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<td>Days refeed</td>
<td>Weight deficit</td>
<td>Days refeed</td>
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<td>11%</td>
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<td>215</td>
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<td>6%</td>
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<tr>
<td>208</td>
<td>38%</td>
<td>0</td>
<td>19%</td>
<td>30</td>
</tr>
<tr>
<td>206</td>
<td>6%</td>
<td></td>
<td>19%</td>
<td>30</td>
</tr>
<tr>
<td>220</td>
<td>24%</td>
<td></td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>168</td>
<td>8%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>231</td>
<td>22%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>223</td>
<td>27%</td>
<td></td>
<td>23%</td>
<td>30</td>
</tr>
<tr>
<td>192</td>
<td>8% above target weight</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>232</td>
<td>15%</td>
<td></td>
<td>0%</td>
<td></td>
</tr>
<tr>
<td>236</td>
<td>23%</td>
<td></td>
<td>0%</td>
<td></td>
</tr>
</tbody>
</table>

* Denotes re-feeding prior to hospital admission.
Table 18

Height, weight and age of anorexia nervosa patients and control subjects.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Weight on admission</th>
<th>Height (Target weight)</th>
<th>Mean weight of population matched for age and height</th>
<th>Age</th>
<th>Duration of present amenorrhoea</th>
<th>Lowest weight ever as % below target weight at that time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>kg</td>
<td>m</td>
<td>kg</td>
<td>yrs</td>
<td>mos</td>
<td></td>
</tr>
<tr>
<td>180</td>
<td>32.0</td>
<td>1.58</td>
<td>49.0</td>
<td>16</td>
<td>91</td>
<td>35</td>
</tr>
<tr>
<td>215</td>
<td>30.6</td>
<td>1.73</td>
<td>58.1</td>
<td>17</td>
<td>58</td>
<td>47</td>
</tr>
<tr>
<td>213a</td>
<td>27.8</td>
<td>1.50</td>
<td>45.4</td>
<td>20</td>
<td>62</td>
<td>39</td>
</tr>
<tr>
<td>211a</td>
<td>39.5</td>
<td>1.60</td>
<td>54.0</td>
<td>23</td>
<td>46</td>
<td>27</td>
</tr>
<tr>
<td>210</td>
<td>45.5</td>
<td>1.65</td>
<td>53.1</td>
<td>15</td>
<td>9</td>
<td>34</td>
</tr>
<tr>
<td>214a</td>
<td>54.3</td>
<td>1.72</td>
<td>60.3</td>
<td>24</td>
<td>48</td>
<td>20</td>
</tr>
<tr>
<td>208</td>
<td>35.8</td>
<td>1.55</td>
<td>49.6</td>
<td>15</td>
<td>21</td>
<td>33</td>
</tr>
<tr>
<td>206a</td>
<td>52.3</td>
<td>1.66</td>
<td>56.4</td>
<td>22</td>
<td>60</td>
<td>27</td>
</tr>
<tr>
<td>229a</td>
<td>39.2</td>
<td>1.65</td>
<td>53.6</td>
<td>19</td>
<td>12</td>
<td>41</td>
</tr>
<tr>
<td>168</td>
<td>52.0</td>
<td>1.63</td>
<td>56.3</td>
<td>25</td>
<td>61</td>
<td>26</td>
</tr>
<tr>
<td>231a</td>
<td>46.9</td>
<td>1.72</td>
<td>60.3</td>
<td>23</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>233a</td>
<td>38.5</td>
<td>1.60</td>
<td>53.1</td>
<td>21</td>
<td>48</td>
<td>30</td>
</tr>
<tr>
<td>192a</td>
<td>60.6</td>
<td>1.68</td>
<td>55.9</td>
<td>17</td>
<td>7</td>
<td>28</td>
</tr>
<tr>
<td>232</td>
<td>44.5</td>
<td>1.58</td>
<td>52.6</td>
<td>19</td>
<td>24</td>
<td>28</td>
</tr>
<tr>
<td>236</td>
<td>40.0</td>
<td>1.65</td>
<td>53.5</td>
<td>20</td>
<td>12</td>
<td>33</td>
</tr>
<tr>
<td>Control subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>52.2</td>
<td>1.57</td>
<td>52.4</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>47.4</td>
<td>1.49</td>
<td>47.6</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C3</td>
<td>56.8</td>
<td>1.65</td>
<td>56.8</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4</td>
<td>60.0</td>
<td>1.73</td>
<td>60.8</td>
<td>19</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C5</td>
<td>63.4</td>
<td>1.73</td>
<td>62.1</td>
<td>21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C6</td>
<td>52.5</td>
<td>1.62</td>
<td>52.7</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a The patients had previously been obese.
present height, and the age at which the patient fell ill using the Documenta Geigy Scientific Tables (1956) as a reference source.

The diet consisted of the normal hospital food, plus biscuits with morning coffee, and either cheese sandwiches or biscuits with tea in the afternoon. The daily energy intake, estimated from weighed food portions and food tables (McCance and Widdowson, 1966) varied from day to day but was generally between 2600 Kcals (10.89 MJ) and 3000 Kcals (12.55 MJ). The average daily intake was 2800 Kcal (11.72 MJ) with 14% of the energy being derived from protein, 36% from fat and 50% from carbohydrate. The patients were carefully supervised and, because they were on strict bedrest and in a unit where staff were expert at managing the condition, there was no opportunity for the patients to hide food or vomit without the knowledge of the nursing staff. The patients ate all the food presented to them and there was no problem with patients vomiting.

None of the patients received medication during the study.

The patients were weighed twice a week. Body surface area was estimated from height and weight using a nomogram derived from the DuBois formula (DuBois and DuBois, 1916).

**Experimental procedure**

The thermic response to a standard meal was measured in all subjects in the resting state by measuring oxygen consumption with a Benedict Roth spirometer. This closed circuit method of indirect calorimetry allows a direct measurement of the volume of oxygen consumed to be made.
Table 19

Contents of Hycal per bottle.

<table>
<thead>
<tr>
<th>Contents</th>
<th>Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>20.57</td>
</tr>
<tr>
<td>Maltose</td>
<td>15.11</td>
</tr>
<tr>
<td>Trisaccharides</td>
<td>12.54</td>
</tr>
<tr>
<td>Tetrasaccharides</td>
<td>10.56</td>
</tr>
<tr>
<td>Pentasaccharides</td>
<td>8.88</td>
</tr>
<tr>
<td>Higher saccharides</td>
<td>38.58</td>
</tr>
<tr>
<td><strong>TOTAL carbohydrate</strong></td>
<td><strong>106.24</strong></td>
</tr>
</tbody>
</table>
Oxygen consumption was calculated from the gradient of the trace. Energy expenditure was estimated from the oxygen consumption corrected for standard temperature and pressure by multiplying with the factor 20.7 for kilojoules and 4.92 for kilocalories.

The meal

The standard meal chosen was Hycal (Beecham Products). One bottle of Hycal was mixed with an equal volume of water. This provides 106 g of carbohydrate and 425 Kcals (1785 KJ). The composition of Hycal is given in Table 19.

The glucose meal was substituted for the midday meal so that the subjects ate breakfast normally and then fasted until the glucose drink was taken at 13.00 hours.

A butterfly needle was inserted into a vein of the forearm so that blood could be collected at frequent intervals. The vein chosen varied between patients, but very often the anterior cubital vein was used. If the anterior cubital vein was used the arm was immobilised. Patients with anorexia nervosa have veins that readily go into venospasm. This sometimes occurred when premeal samples were taken. When this happened the arm was gently warmed with a hot water bottle. Blood samples were withdrawn by syringe and the cannula was kept patent with heparinised saline. Six millilitre blood samples were taken, one millilitre was placed in a fluoride coated pot for blood glucose determination, then the remaining 5 millilitres was placed in a heparinised tube with 0.5 ml of Trasylol (500,000 U/ml) mixed gently by rocking the tube a few times then centrifuged immediately for five minutes. The plasma was removed with a
Figure 22: Experimental plan for the anorexia nervosa study. Hatched areas indicate the periods when oxygen consumption was measured. The arrows indicate when blood samples were taken.
Pasteur pipette and aliquoted into several small polythene tubes, frozen immediately and stored at -20°C. Each time an assay was to be performed one tube could be removed so that repeated thawing and refreezing was avoided. Plasma samples were assayed for glucagon and insulin. Details of the assays are given in Appendix A.

The subject lay comfortably at rest and breathed through the spirometer. Each subject practised several times in order to become familiar with the apparatus. The experiment began with two ten minute measurements of oxygen consumption, with a five minute interval between them to determine a baseline or premeal resting energy expenditure. The subject then drank the Hycal over a five minute period. Ten minute recordings of oxygen consumption were made every fifteen minutes for two and a half hours after ingestion of the drink. The plan of the experiment is shown in Figure 22.

Five of the anorectic patients (180, 208, 211, 214, 223) were tested twice during their treatment at intervals of one month. Patient 213 was tested on three occasions and patient 215 on four occasions. Details of the percentage below target weight and the extent of refeeding of the patients on each test occasion are given in Table 17.

**RESULTS**

**Weight gain of anorexia nervosa patients during rehabilitation**

The weight histories of the anorectics are shown in the weight gain curves in Figure 23 and Table 20. The average weight gain was 167 g/day. Most of the patients gained weight faster during the first ten days after admission than later. Average gain during the first ten days was
Figure 23: Weight curves of anorexia nervosa patients during refeeding. Upper set of curves are those of eight previously obese patients (PO). Lower set of curves are those of seven previously normal weight patients (NO). The arrows denote the target weight for that patient.
Table 20

Weight histories of anorexia nervosa patients.

<table>
<thead>
<tr>
<th>Patient</th>
<th>% Below target weight on admission</th>
<th>Weight gain first 10 days</th>
<th>Weight gain second 10 days</th>
<th>Weight gain last 10 days before target weight reached</th>
<th>Days taken to reach target weight</th>
<th>Overall weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>35</td>
<td>330</td>
<td>220</td>
<td>240</td>
<td>113</td>
<td>150</td>
</tr>
<tr>
<td>215</td>
<td>47</td>
<td>220</td>
<td>60</td>
<td>70</td>
<td>229</td>
<td>120</td>
</tr>
<tr>
<td>213</td>
<td>39</td>
<td>430</td>
<td>150</td>
<td>160</td>
<td>95</td>
<td>185</td>
</tr>
<tr>
<td>211</td>
<td>27</td>
<td>424</td>
<td>230</td>
<td>230</td>
<td>74</td>
<td>200</td>
</tr>
<tr>
<td>210</td>
<td>14</td>
<td>340</td>
<td>70</td>
<td>160</td>
<td>49</td>
<td>155</td>
</tr>
<tr>
<td>214</td>
<td>10</td>
<td>300</td>
<td>150</td>
<td>210</td>
<td>27</td>
<td>222</td>
</tr>
<tr>
<td>218</td>
<td>28</td>
<td>300</td>
<td>90</td>
<td>150</td>
<td>95</td>
<td>145</td>
</tr>
<tr>
<td>221</td>
<td>7</td>
<td>130</td>
<td>240</td>
<td>190</td>
<td>25</td>
<td>164</td>
</tr>
<tr>
<td>225</td>
<td>27</td>
<td>300</td>
<td>180</td>
<td>150</td>
<td>96</td>
<td>150</td>
</tr>
<tr>
<td>168</td>
<td>8</td>
<td>120</td>
<td>200</td>
<td>260</td>
<td>24</td>
<td>180</td>
</tr>
<tr>
<td>231</td>
<td>22</td>
<td>210</td>
<td>240</td>
<td>183</td>
<td>67</td>
<td>200</td>
</tr>
<tr>
<td>223</td>
<td>28</td>
<td>200</td>
<td>80</td>
<td>α</td>
<td>α</td>
<td>184</td>
</tr>
<tr>
<td>192</td>
<td>8% above target weight</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>142</td>
</tr>
<tr>
<td>232</td>
<td>15</td>
<td>200</td>
<td>130</td>
<td>α</td>
<td>α</td>
<td>144</td>
</tr>
<tr>
<td>236</td>
<td>25</td>
<td>200</td>
<td>130</td>
<td>α</td>
<td>α</td>
<td></td>
</tr>
</tbody>
</table>

α These patients did not reach target weight during the study.
Table 21

Weight gain during treatment of eight anorexia nervosa patients who had been previously obese (PO group) and seven anorexia nervosa patients with no history of obesity (NO group).

<table>
<thead>
<tr>
<th></th>
<th>First 10 days</th>
<th>Second 10 days</th>
<th>Last 10 days before target weight reached</th>
<th>Overall weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO group</td>
<td>299</td>
<td>181</td>
<td>187</td>
<td>187</td>
</tr>
<tr>
<td>NO group</td>
<td>244</td>
<td>129</td>
<td>176</td>
<td>148</td>
</tr>
<tr>
<td>T test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>
272 g/day. This was significantly greater (p < 0.005) than during the second ten days (average 155 g/day) or the overall weight gain. There was no significant difference between weight gain during the second ten days after treatment began and weight gain during the last ten day period before target weight was reached (average 182 g/day).

Because of the wide range of overall weight gain (120-222 g/day) in patients receiving the same food intake and restricted physical activity we decided to test whether patients who were previously obese (PO) regained weight more quickly than those who had no history of obesity (NO) (Table 21). In the first ten days the average weight gain of the PO patients was 299 g/day and the NO patients 244 g/day. For the second ten days the weight gains were 181 g/day and 129 g/day respectively and in the last ten days before target weight was reached, the PO patients gained 187 g/day and the NO 176 g/day, but these differences between the weight gains of the PO and NO patients do not reach statistical significance. However, overall the PO patients gained on average 187 g/day, and the NO patients, 148 g/day. This difference is statistically significant (p < 0.01). There was no significant difference between the average weight deficit, on admission, of the PO and NO groups.

Resting metabolic rate

The energy expenditure of the anorectic patients was lower than that of the control subjects, both before and after the glucose meal, when expressed either as Kcal/hour or as Kcal/square metre surface area/hour. It was also lower before the meal, but higher after the meal when expressed as Kcal/kg body weight/hour (Figure 24). Energy expenditure increased after the glucose meal in both the anorectic and control groups but the percentage increase was much greater in the anorectic group (p < 0.005) (Figure 25).
Figure 24: Metabolic rate before and after a glucose meal expressed in three different ways. Left to right Kcal/h; Kcal/m²/h; Kcal/kg body weight/h.

Anorectics ........ (25 tests)

Control group ------ (6 tests)

100g glucose by mouth
Figure 25: Thermic effect of 100 g glucose meal.

Mean ± S.E. in 15 anorectic and six control subjects.
Table 22

Changes in resting metabolic rate with treatment in anorexia nervosa patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Kcal/hr</th>
<th>Kcal/m²/hr</th>
<th>Kcal/kg/hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early treatment group</td>
<td>46 ± 2.5</td>
<td>33 ± 1.6</td>
<td>1.1 ± 0.06</td>
</tr>
<tr>
<td>Late treatment group</td>
<td>50 ± 1.3</td>
<td>38 ± 1.0</td>
<td>1.1 ± 0.04</td>
</tr>
<tr>
<td>T test</td>
<td>p &lt; 0.001</td>
<td>p &lt; 0.01</td>
<td>NS×</td>
</tr>
<tr>
<td>Early treatment PO patients</td>
<td>50 ± 3.8</td>
<td>36 ± 2.3</td>
<td>1.2 ± 0.07</td>
</tr>
<tr>
<td>Early treatment NO patients</td>
<td>42 ± 1.8</td>
<td>31 ± 1.6</td>
<td>1.1 ± 0.10</td>
</tr>
<tr>
<td>T test</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Late treatment PO patients</td>
<td>56 ± 1.3</td>
<td>36 ± 0.8</td>
<td>1.0 ± 0.04</td>
</tr>
<tr>
<td>Late treatment NO patients</td>
<td>62 ± 1.5</td>
<td>40 ± 1.1</td>
<td>1.2 ± 0.04</td>
</tr>
<tr>
<td>T test</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
<td>p &lt; 0.05</td>
</tr>
</tbody>
</table>

α NS, not significant.
For the purpose of analysis the anorectics were divided into (1) an "early treatment" group who were grossly underweight (15-42%) and had been refeed for 34 days or less, and (2) a "late treatment" group who were within 10% of their target weight and had eaten a normal diet for at least four weeks. The figure 34 days refeeding was chosen in order to include the first test occasion on patient 215 who, although she had been refeed for over a month, could still be regarded as early in treatment because of her large weight deficit, 42%. Five test occasions are not classified in either group but are included in the anorectic group as a whole. The premeal resting metabolic rate (RMR) in the anorectics increased with treatment when expressed as Kcal/hour or per unit surface area (Kcal/m^2/hour) but not when expressed as Kcal/kg body weight/hour. The changes in resting energy expenditure brought about by treatment are summarised in Table 22.

Although the premeal metabolic rate of the late treatment group as a whole was lower (p < 0.001) than that of the control group - both in gross terms and when related to surface area - there was no significant difference between the premeal metabolic rate of the late treatment NO group and the controls.

Thermic response to glucose

The thermic response to glucose was measured by plotting the metabolic rate after the meal against time, dissecting the area under the curve and weighing it. The thermic responses were compared in the different groups (Table 23). In the anorectic group the average increase in metabolic rate over the 150 minutes was 16% and this was significantly greater (p < 0.005) than the average increase of 5% observed in the controls. The previously obese anorectics had an average increase in thermic response of 14% and the previously non-obese anorectics an increase of 19%. This
Table 23
Thermic response to a glucose meal in anorexia nervosa patients and control subjects.

<table>
<thead>
<tr>
<th></th>
<th>Anorectic group</th>
<th>Control group</th>
<th>T test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average % increase in metabolic rate</td>
<td>16</td>
<td>5</td>
<td>p &lt; 0.005</td>
</tr>
<tr>
<td>Actual increase in energy expenditure (Kcal)</td>
<td>19</td>
<td>8</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

PO anorectics          NO anorectics

<table>
<thead>
<tr>
<th></th>
<th>Average % increase in metabolic rate</th>
<th>14</th>
<th>19</th>
<th>NS p &lt; 0.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual increase in energy expenditure (Kcal)</td>
<td>18</td>
<td>21</td>
<td></td>
<td>NS p &lt; 0.70</td>
</tr>
</tbody>
</table>

Early treatment anorectics Late treatment anorectics

<table>
<thead>
<tr>
<th></th>
<th>Average % increase in metabolic rate</th>
<th>14</th>
<th>17</th>
<th>NS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual increase in energy expenditure (Kcal)</td>
<td>18</td>
<td>23</td>
<td></td>
<td>NS p &lt; 0.20</td>
</tr>
</tbody>
</table>
difference is not significant (p < 0.3).

The difference between the average increase in metabolic rate after oral glucose at different stages of treatment, namely 14% for the early treatment group and 17% for late treatment groups was not significant (p < 0.5). These figures represent an average increase in actual energy expenditure during the 2½ hours after oral glucose, of 19 Kcal for the anorectic group and 8 Kcal for the control group; 18 Kcal for the PO and 21 Kcal for the NO anorectics; 15 Kcal for the early treatment group and 23 Kcal for the late treatment group. The difference in actual energy expenditure between the anorectic and control group was significant (p < 0.01) but the differences in actual energy expenditure between the PO anorectics and NO anorectics, and between the early and late treatment anorectics were not (p < 0.70 and p < 0.20 respectively).

There was a tendency in the early treatment group for the thermic effect to be inversely related to the premeal metabolic rate, that is those patients with the lowest premeal metabolic rate tended to have the largest thermic effect. The same tendency was not found in the late treatment group, or the control group.

For the control group
\[ r = -0.01 \] relationship not significant

For the early treatment group
\[ r = -0.82 \quad p < 0.02 \]

For the late treatment group
\[ r = -0.40 \] relationship not significant.
Comparison of premeal resting metabolic rate with basal metabolic rate standards

Although the experiments were deliberately not performed under basal metabolic rate (BMR) conditions it is interesting to compare the results with standards for BMR. Those of Robertson and Reid (1952) were chosen for comparison because they represent measurements made on a similar population to our own. As might have been anticipated the premeal resting metabolic rate of the control group was, on average, 20% above BMR standards.

The Robertson Reid standards are given as metabolic rate in Kcal/m²/hour for persons of a particular age.

There are two ways in which the premeal resting metabolic rate of the anorectics can be compared with the standards, either:

1. A direct comparison can be made between the premeal resting metabolic rate expressed as Kcal/m²/hour and the standard for a person of the same age and sex, or

2. the target BMR can be estimated for the patient at her target weight. From the DuBois nomogram the target surface area of the patient can be derived from her height and target weight and then using the BMR standard for a person of the same age and sex the target BMR can be calculated.

Using the first method the anorectics who were being refed had a resting metabolic rate on average 5% above the BMR standards, but by the second method they were 0.2% below the BMR standards. Five patients had particularly low metabolic rates, 208, 223, 223, 232 and 236. This was because they were all tested within 3 days of admission and before any
Comparison of premeal resting metabolic rate with basal metabolic rate standards.

<table>
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<th>Premeal resting metabolic rate as % difference from BMR standard (Robertson &amp; Reid, 1952)</th>
<th>Premeal metabolic rate as % difference from target BMR</th>
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<tr>
<td>236</td>
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<td>- 11</td>
<td>- 20*</td>
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</tbody>
</table>

* Admission group
substantial re-feeding. Patients 208, 223, 232 and 236 were in fact
tested immediately on admission. These 5 patients are subsequently called
the admission group. Their resting metabolic rate was on average 15% below
the standards for BMR when expressed as Kcals/m²/hour but 25% below the
BMR standard for a person the same age and height but the target weight
of the patient.

A detailed comparison of the two methods for the anorectic
patients is given in Table 24.

DISCUSSION

The traditional view of many nutritionists is that people who
eat the same quantity of food energy and perform the same amount of
physical work or activity will maintain the same body weight and that if
food intake is increased for every extra 9 kilocalories consumed 1 g of
fat will be laid down. However, it is becoming clearer from both
epidemiological evidence and human experimentation that it is unlikely
that this is true. The confusion exists
partly because of the difficulty of measuring accurately food energy
intake and energy expenditure.

Cross-sectional dietary surveys of food intake, invariably show
little correlation between body weight and food intake. Studies which compare
food intake with energy expenditure also tend to show little or no
correlation between food intake and energy expenditure. Edholm's careful
study (1955) on 12 army cadets found that there was no correlation between
either DAILY energy intake and DAILY energy expenditure, or energy intake
and expenditure over the whole 14-day period summed for each individual, so
that any regulation of energy balance in man must be a long term control.
Garrow (1974) points out in his critical review that although in Edholm's study the MEAN energy intake of all the cadets for the 14-day observation was 3432 Kcal/day and the MEAN energy expenditure was 3416 Kcal/day, this is not evidence that any individual cadet balances energy input and output over a 2-week period.

Another approach to the investigation of how man achieves energy balance is to look at the weight changes of individuals over long periods of time. Very few such longitudinal studies of adults have been published; we have to rely instead on cross sectional studies of different age groups. This may be misleading the average values disguising the individual changes. Garrow (1974) looked at weight changes in long-stay prisoners and found that on average their weight varied over a range of 7.5 kg during a period of 7 years and Gordon and Kannel (1973) showed that the adult population of Framingham had average weight fluctuations of 10 kg over a period of 18 years. Garrow suggests that such studies may be interpreted as 'high precision long term regulation' because a weight change of 10 kg over such a long period represents a very small percentage of energy intake for that time. Assuming an average daily intake of 2500 Kcal, and the energy value of the 10 kg of weight gain to be 60,000 Kcal, it represents an error of about 0.3% or about 7.5 Kcal/day. This error is certainly smaller than the experimental error in the determination of food energy intake or expenditure. There is thus some evidence that the regulation of energy balance in the short term is imprecise but that in the long term a finer control is achieved. This long term control is not necessarily by a physiological mechanism; as Garrow points out a weight change of 10 kg is noticeable to the individual who may respond to social and psychological pressures to control his weight gain by altering food intake, energy expenditure or both.
There is good evidence in the literature discussed fully in the introduction to this thesis that short term energy balance is affected not only by changes in food intake where this is possible, but by reduced metabolic rate when food is restricted and elevated metabolic rate when food intake is increased.

If energy balance is regulated either in the short term or long term it should be possible to distinguish between individuals who have good regulation of energy balance and those in whom the balance mechanism is defective.

In the present study it was found that when the anorectics were classified according to their previous weight, those who were previously obese gained weight more quickly than those who were previously of normal weight. The previously obese patients had possibly some degree of fat cell hyperplasia as has been demonstrated in obesity. It has often been suggested that fat cell hyperplasia leads to obesity but the question remains how does the fat cell hyperplasia change the energy balance equation? Does the number of fat cells alter the food intake? the energy expenditure? or the efficiency with which food energy is used? or perhaps even a combination of these possibilities?

It is necessary to consider three possible reasons why the previously obese anorectic patients gained weight more quickly than the previously non-obese patients.

1. They ate more food.
2. They had lower metabolic rates throughout the day.
3. The available or metabolisable energy of the diet was greater for the previously obese than the previously non-obese patients.
It is possible, but because of the strict supervision of the patients, unlikely, that the PO patients ate more food than the NO. The patients themselves were attentive to the uniformity of the treatment programme and watched carefully to see they were given the same amounts of food as other patients.

Collections of urine and faeces were not made so that it is not possible to examine whether the digestible or metabolisable energy of the diet was different for the two groups. However, the difference in growth rates between the groups corresponds to a loss of at least 273 Kcal per day and so it is unlikely that the difference in growth rate can be accounted for in this way.

The suggestion remains that energy expenditure was less in the previously obese. A difference in energy expenditure could have been due to different levels of activity, resting metabolic rate or the thermic effect of food. The range of activity permitted was very restricted because the patients were on bed rest until they achieved their target weight. However, as some of the patients had consciously increased activity during their illness to achieve greater weight loss it was thought worthwhile to test whether these patients gained weight more slowly because they were more active in bed. There was little evidence from the weight gain data that this was so. The activity prone patients (213, 211, 210, 208, 223, 232 and 236) gained on average 165 g/day and the rest 173 g/day but the difference is not statistically significant.

A direct comparison between energy expenditure per unit time in the groups cannot be made because the two groups were not matched at the times when measurements were taken for degree of emaciation or extent of re-feeding. As shown in the results (p.110) the resting metabolic rate was
much lower early in the treatment. Since food intake was constant throughout, the energy surplus of food intake over expenditure was necessarily greater at the commencement of treatment than towards the end. This may partly explain the faster weight gains in the first ten days, although in some patients exceptionally high weight gain was undoubtedly due to restoration of hydration and the introduction of carbohydrate to the diet, Pilkington, et al. (1980). This was shown most dramatically by patient 213 who had restricted her fluid intake as well as food prior to admission and gained 530 g/day in the first ten days of treatment.

The difference in weight gain between the PO and NO patients could not be explained by differences in salt and water retention because the weight gain in the first 10 days, which should reflect this, was not significantly different between the two groups, and the PO patients consistently gained slightly more weight throughout their treatment. Further support that water retention was not an important factor was the observation that the weight gain in the first ten days represented only a small proportion of the total weight gain.

The anorectic patients demonstrated the well-documented reduction in metabolic rate which is associated with and caused by under-nutrition (Benedict, et al., 1919; Keys, et al., 1950; Apfelbaum, et al., 1971; Crisp, 1970). In the newly admitted patients this amounted to a 24% reduction in gross terms or a 15% reduction in relation to surface area. From this it can be deduced that 37%, i.e. $\frac{24-15}{24} \times 100$ of the reduction in RMR is a direct consequence of the low weight and the remaining 63% of the reduction in RMR is a result of economy of energy utilisation brought about by a
reduction in metabolic rate per unit of body tissue weight. It should be stressed that this comparison is between RMR of anorectics at midday and standard values for BMR and consequently the difference between true basal metabolic rate of the anorectics and the BMR standards may have been even larger.

The average weight deficit of the newly admitted anorectic patients was 23% (range 15-28%) which is similar to that produced by Keys and his co-workers (1950), who deliberately restricted food intake in their male human volunteers with the intention of producing a 25% weight deficit. In their volunteers the BMR deficit was 38.89% in gross terms, but only 31.26% per square metre of body surface area from which it can be computed that 20% of the reduction in BMR was due to loss of tissue and 80% to economy of energy utilisation.

Taylor and Keys (1950) published a further paper on the adaptation to calorie restriction in the Minnesota experiment in which they suggest that 'the greater part of the decrease in the BMR (65%) in starvation reflects the shrinkage of the metabolising mass of tissue, and the smaller part (35%) should be ascribed to a decrease in the intensity of metabolism'. It will be noted that these values are contrary to my own calculations from his data given above; why the discrepancy?

They calculated metabolising or 'active' tissue as the body weight minus the weight of bone mineral (estimated as 4% of original body weight), extracellular fluid (thiocyanate space), plasma volume (blue dye), and fat (specific gravity method, under water weighing). By these methods they found that the percentage loss of active tissue was greater than the
percentage loss of body weight. This is a surprising finding especially in the light of his statement elsewhere (p.176 Human Starvation) that the body fat decreased more markedly than the muscle mass, estimated from the cross sectional area of the upper arm and thigh. Keys also states both in Human Starvation, p.329, and his 1950 paper that the deficit in BMR was 19.3% per kilogram body mass and 15.5% per kilogram active tissue. This is impossible unless there is a net oxygen consumption outside the 'active' metabolising tissue, which decreased more than the decrease of the 'active' tissue, an interesting but unlikely possibility. Grande (1958), jointly with Anderson and Keys, in a further study of semi-starvation in men found that between 27% and 35% of the decrease in basal metabolism could be explained by loss of 'cells' and 65-73% of the decrease could be attributed to an actual decline in metabolic rate of the 'cells' remaining in the body. In this case they had determined what they called 'cells' from determinations of total body water and extracellular fluid and nitrogen loss over the starvation period.

They noted the discrepancy with Keys' earlier publication but suggested the difference was because the semistarvation in Keys' first study was prolonged (24 weeks) and the later study much shorter (24 days); they suggested that the change in basal metabolism takes place in two different phases. In the first phase the decrease in basal metabolism exceeds the loss of 'cells' in the body and in the second phase the metabolic rate per weight unit of 'cells' remains constant but there is loss of 'cells'. However, this suggestion is not consistent with the finding that nitrogen loss is greater at the beginning of periods of food restriction and then gradually decreases as the food restriction continues.
The probable explanation for the difference between the Minnesota experiment and the later study is that the techniques for estimating body composition were improved in the later study.

There was a 17.1% decrease in basal metabolism as Kcal/hour and a 13.4% decrease when expressed per square meter of body surface in the later study (Grande, et al., 1958). Using the method described earlier $(17.1 - 13.4) \times 100/17.1$, 21% can be said to be due to loss of weight and $13.4 \times 100/17.1$ 79% due to reduced metabolic rate per unit mass of tissue. These figures compare well with Grande's own using body composition data (27-35% due to loss of 'cells' and 65-73% due to decline of metabolic rate of the 'cells' and suggest that surface area is quite a good measure of lean body mass.

It is also worth noting that Grande, Anderson and Keys (1958) found that the BMR deficit per kilogram body weight was 8.7% and per kilogram of cells 11.8%, results which are consistent with each other and rather different from Keys' earlier finding. Unfortunately, Garrow (1974) quotes Keys' early paper and suggests that the greater part of the economy of food utilisation that occurs in semi-starvation can be accounted for by loss of lean body mass.

There were 2 PO and 3 NO patients amongst the five newly admitted anorectics investigated in the present study. The total deficit in RMR was 21% in the 2 PO subjects; or 11% if expressed on the basis of surface area. In the case of the 3 NO subjects the total deficit in RMR was 26% or 18% on a surface area basis. Thus, for the PO 52% of the reduction in RMR, compared with average BMR standard, was due to economy of energy utilisation.
The corresponding figure for the NO anorectics was 69%. From this it appears the NO anorectic patients adapt better to food shortage for they achieve a reduced metabolic rate more by economy of food utilisation than by loss of body tissue.

The NO patients also adapted better to a plentiful supply of food. As treatment progressed they increased their metabolic rate more than the PO group who might reasonably, therefore, show a greater tendency to become obese once more.

The late treatment NO anorectics' metabolic rate was not significantly lower than that for the control group, whereas the metabolic rate of the late treatment PO anorectics was significantly lower as Kcal/h, Kcal/m²/h and Kcal/kg/h. This lower metabolic rate may be because the PO have a smaller lean body mass or a lean body mass with a lower metabolic rate per unit weight. The finding that the metabolic rate is lower per kilogram body weight even late in treatment suggests that the body composition of this group of patients may be fatter. Whatever the reasons for the lower RMR and because of the relatively large proportion of daily energy expenditure it represents (Garrow, 1974), this population may be predicted as one that will have difficulty in maintaining 'normal' weight. This finding makes it more easy to understand and hence to explain the frustration of the anorectic who tends to become obese on an equal or even lower food intake than her slim friends.

The main aims of this experimentation were to see if the thermic effect of food was greater in anorectics than in a normal population, and to test if the size of the thermic effect was related to the rate of weight gain of the patients as had been found by Brooks and Ashworth (1972)
in malnourished infants. The large thermic response to glucose, amounting to a 16% increase in resting metabolic rate, which was found in the anorectic patients and which persisted for 2.5 h is of similar magnitude to that found in malnourished infants by Brooke and Ashworth (1972) who reported a 13% increase in metabolic rate for 1.75 hours after a mixed nutrient meal. Contrary to Brooke and Ashworth, however, we found no correlation between the rate of weight gain and the size of the thermic effect. If anything, our late treatment anorectics - who were either stationary or gaining weight slowly - tended to have a larger thermic response to glucose than either the early or mid-treatment patients. Moreover, those patients who gained weight most quickly, i.e. the PO group, had in general the small thermic response to oral glucose.

The possibility that the relatively large thermic response to glucose shown by anorectic patients as a whole could have been due to anxiety occasioned by the ingestion of a large dose of carbohydrate cannot be completely dismissed although there was no visible evidence during the tests that this was so. Green, et al. (1975) have also reported a large thermic effect of food in anorectic patients. They described a 30% increase in oxygen consumption following a 'standard' breakfast in six anorectic patients.

The thermic response to oral glucose was smaller in the control subjects than was reported by Pittet, et al. (1974) in seven male students in whom oral glucose produced a 14% increase in metabolic rate over a 2.5 h period. Gomez, et al. (1972) observed an 11% increase in metabolic rate during three hours after a 100 g glucose meal. Garrow, et al. (1972), on the other hand, reported a 6.6% increase in metabolic rate during 3.5 hours after 160 g sucrose but an approximately 2% increase after a meal
containing only half that amount of sucrose. He commented that the seemingly low increase of 6.6% in metabolic rate evoked by the large dose of sucrose might have been due to the high baseline metabolic rate before the meal. This could also be true for the control subjects of this study.

CONCLUSION

These results confirm those of Ashworth (1969) that the thermic effect of food is large during weight gain but in contrast to her findings no evidence of a correlation between the rate of weight gain and the size of the thermic effect is found in the present study. The anorectics in this study gained weight because energy intake, in the form of food, exceeded the rate of energy expenditure. This was compounded by the overall low metabolic rate in the anorectic patients both in the premeal and fed state. Previously obese anorectics gained weight even faster than the previously non-obese, probably because of their smaller increase in metabolic rate with rising weight and their tendency to exhibit a smaller thermic response to food.
CHAPTER FOUR

GLUCOSE TOLERANCE AND PLASMA INSULIN LEVELS DURING RECOVERY FROM
ANOREXIA NERVOSA
CHAPTER 4

GLUCOSE TOLERANCE AND PLASMA INSULIN LEVELS DURING RECOVERY FROM ANOREXIA NERVOSA

Introduction

Results

First analysis - stage of treatment and glucose tolerance
- previous obesity and glucose tolerance
- duration of illness and glucose tolerance

Second analysis - comparison between glucose tolerance in the anorexia nervosa patients and the control group using computer programme RSK4

Third analysis - stepwise multiple regression analysis

Discussion
GLUCOSE TOLERANCE AND PLASMA INSULIN LEVELS DURING RECOVERY FROM ANOREXIA NERVOSA.

INTRODUCTION

As the meal chosen for the thermogenesis experiments was a starch hydrolysate frequently used in studies of glucose tolerance it was possible to study carbohydrate metabolism concurrently with the investigation of dietary thermogenesis. A description of the collection and analysis of the blood samples is given in Chapter three of this thesis.

The results of the glucose tolerance tests were treated consecutively in three ways.

First analysis

The tests were divided according to (i) stage of treatment, (ii) previous obesity and (iii) duration of illness, and compared.

Second analysis

The glucose and insulin curves were characterised using a computer programme, and a comparison made between early and late treatment anorexia nervosa patients and the control group.

Third analysis

A stepwise multiple regression analysis was used to investigate which 'characteristics' of this heterogeneous population of anorexia nervosa patients influenced glucose tolerance the most.

RESULTS - First analysis

Stage of treatment and glucose tolerance

The tests were divided into (1) an 'early treatment' group of patients who were grossly underweight (15-12%) and had been re-fed for 34 days or less and (2) a 'late treatment' group of patients who were
Figure 26: Blood glucose and plasma insulin levels after 100 g of glucose by mouth in 5 female anorexia nervosa patients on admission and prior to refeeding; mean ± S.E.
within 10% of their target weight and had eaten a normal diet for at least four weeks. Five patients, tested within 3 days of admission, were called the admission group.

Glucose tolerance and the insulin response to an oral glucose load were normal in the admission group (Figure 26).

As treatment progressed and the patients gained weight glucose tolerance became impaired, insulin levels increased and peak insulin levels occurred later (Figure 27).

Previous obesity and glucose tolerance

A patient was classified as having been previously obese if, at anytime, they had been more than 15% above their target weight for their height and age. The tests were divided into those from patients who had been previously obese and those with no history of obesity. Basal glucose levels were lower in the previously obese patients (p < 0.05) but there were no other statistically significant differences in the glucose or insulin levels after oral glucose although there was a tendency for insulin levels to be lower in the previously obese patients, (Figure 28).

Duration of illness and glucose tolerance

Patients who had suffered from anorexia nervosa for more than 4 years were classified as long duration of illness. The tests from these patients were compared with those from the patients who had been anorectic for less than four years (Figure 29). The blood glucose and plasma insulin values were not significantly different either before or after the oral glucose, but there was a tendency for the long duration patients to have more impaired glucose tolerance.
Figure 2: (see legend on separate page)

- 100 g glucose by mouth
- Early treatment
- Late treatment
- Plasma insulin (IU/ml)
- Blood glucose (mg/100 ml)

(minutes)
Figure 27 Legend:

Blood glucose and plasma insulin levels after 100 g of glucose by mouth in 8 early treatment and 8 late treatment female anorexia nervosa patients. The mean ± S.E. represent 10 tests in the early treatment group and 12 tests in the late treatment group. Glucose and insulin levels in the two groups were significantly different in each sample after the oral glucose load p < 0.05.
Figure 28: (see Legend on separate sheet)
Figure 28 Legend:

Blood glucose and plasma insulin levels in 8 previously obese anorexia nervosa patients (PO) and in 6 anorexia nervosa patients with no history of obesity (NO). The mean ± S.E. represent 13 tests in the PO group and 12 tests in the NO group.
Blood glucose
mg/100 ml

Plasma, insulin
µU/ml

Figure 29: (see Legend on separate sheet)
Figure 29 Legend:

Blood glucose and plasma insulin levels in 7 anorexia nervosa patients who had been ill for more than four years (long duration) and 7 anorexia nervosa patients who had been ill for less than four years (short duration). The mean ± S.E. represent 15 tests in the long duration group and 10 tests in the short duration group.
Figure 30: Blood glucose and plasma insulin levels in an anorexia nervosa patient (210) after 100 g of glucose by mouth. This patient had been refed for 78 days before the test and was at target weight.

Blood glucose  Plasma insulin
mg/100 ml  µU/ml

---

0 30 60 90 120 150
mins

glucose
insulin
100 g glucose by mouth
There was considerable individual variation in the levels of glucose, insulin and the timing of the peak responses, which is reflected in the large standard errors and low levels of statistical significance. Some examples of the individual variations are illustrated in Figures 30, 31 and 32.

Examples

Patient 210; had a normal response to oral glucose (peak blood sugar level and two hour blood sugar less than 161 mg/100 ml and 121 mg/100 ml respectively). However she had very high insulin levels at 60, 75, 90 and 120 minutes after oral glucose, (Figure 30).

Patient 215; had a slightly impaired glucose tolerance on a test performed 34 days after admission when she was still 42% below target weight. 177 days later, when she was 2% below target weight, glucose tolerance was even more impaired. Insulin levels were higher, particularly at 90, 120 and 150 minutes when values of 200, 250 and 250 μu/ml were observed, (Figure 31).

Patient 213; showed a pattern of increasingly impaired glucose tolerance during refeeding and weight gain, but in this case the impaired glucose tolerance was associated with low insulin levels until, on the third test, the 120 and 150 minute values (52 and 65 μu/ml) were much higher than had previously been observed. On each occasion the insulin peak was one hour after the glucose peak, (Figure 32).
Figure 31: Blood glucose and plasma insulin levels in an anorexia nervosa patient (215), after 100 g of glucose by mouth. (Two tests).
Figure 32: Blood glucose and plasma insulin levels in an anorexia nervosa patient (213) after 100 g of glucose by mouth. (Three tests).
Test (1) was after 25 days refeeding she was 23% below target weight
(2) was after 53 days refeeding she was 14% below target weight
(4) was after 205 days refeeding she was at target weight.
Figure 33: Blood glucose and plasma insulin levels in an anorexia nervosa patient (208) on admission prior to any refeeding and after one month's refeeding.
Patient 208; was given a glucose tolerance test immediately on admission without any prior refeeding. The results are shown in Figure 33. Glucose tolerance was normal and the insulin peak was just 15 minutes after the glucose peak. After one months refeeding glucose tolerance was slightly impaired, the peak glucose occurred 30 minutes later, and the highest insulin level was the 150 minute value; 75 minutes after the glucose peak.

Three patients (211, 180 and 213) had a pathological response to oral glucose (Figure 34) according to the classification suggested by the European Diabetes Epidemiology Study Group (Jarrett, 1970); that is, a peak value of more than 220 mg/100 ml and a 2 hour value of more than 150 mg/100 ml. Patients 211 and 180 were 5% and 4% below target weight and patient 213 was at target weight. They had been refed for 55, 105 and 205 days respectively, (Figure 34).

Second analysis - Comparison between glucose tolerance in the anorexia nervosa patients and the control group.

In an attempt to characterise the glucose and insulin responses more rigourously a computer programme, RSK 4, was devised which calculated the mean glucose level from 0 to 60 minutes, the mean glucose level from 0 to 120, the mean glucose 60 to 120 minutes, and the ratio of the area under the curve 60 to 120 minutes over the area 0 to 60 minutes (subsequently called the 2 hour/1 hour ratio). Similar parameters were derived for the insulin curves. The computer programme was developed by the Brendon McGuinness St. Georges Hospital and is given in Appendix 3.

The abbreviations adopted for the parameters were as follows:-
Figure 34: Blood glucose and plasma insulin levels in three anorexia nervosa patients (211, 180, 213)

Blood glucose mg/100 ml

Plasma insulin μU/ml

- - - glucose. Patient 180 re-fed 105 days, 4% below target weight
- - - insulin.

- - - glucose. Patient 211 re-fed 55 days, 8% below target weight
- - - insulin.

- - - glucose. Patient 213 re-fed 205 days, at target weight
- - - insulin.
Prefix   G   Glucose  
I   Insulin  
Suffix  B   Basal (mean of 2 resting levels)  
P   Peak (above origin)  
Pd   Peak (above basal)  
60   0-60 min weighted mean (above origin)  
60D   0-60 min weighted mean (above basal)  
120   0-120 min weighted mean (above origin)  
120D   0-120 min weighted mean (above basal)  
120E   60-120 min weighted mean (above basal)  
RT   2 hr/1 hr ratio (e.g. GRT = G120E/G60D)  

Weighted mean was defined as area under the curve/time.

In this analysis seven early treatment patients were compared with seven late treatment patients and with the control group. Patients were only included in one group, that is either the early or the late treatment group, even though some patients had been tested both early and late in treatment.

The results are summarised in Table 25. No statistically significant differences were found for any of the parameters between the early treatment and the control groups. In comparison, the late treatment group showed impaired glucose tolerance and a sustained insulin response, as indicated by the peak glucose and insulin levels and the areas under the curves.

Although these results indicated a general trend towards impaired glucose tolerance and increased insulin resistance as the patients were refed and gained weight, there remained wide variation between individuals within the groups. Thus, it was decided to examine whether other factors such as previous obesity, bulimia, vomiting activity and purging which contributed to the heterogeneity of the group influenced the results.
Table 25  Parameters derived from the computer programme RSK4  

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<td>112 (± 5)</td>
<td>148 (± 9)</td>
<td>N.S.</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>G120</td>
<td>134 (± 4)</td>
<td>117 (± 8)</td>
<td>160 (± 14)</td>
<td>N.S.</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>IB</td>
<td>1 (± 0.5)</td>
<td>6 (± 2)</td>
<td>5 (± 2)</td>
<td>N.S.</td>
<td>N.S</td>
</tr>
<tr>
<td>IP</td>
<td>50 (± 6)</td>
<td>58 (± 8)</td>
<td>151 (± 33)</td>
<td>N.S.</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>I60</td>
<td>23 (± 4)</td>
<td>30 (± 4)</td>
<td>43 (± 11)</td>
<td>N.S.</td>
<td>N.S</td>
</tr>
<tr>
<td>I120</td>
<td>33 (± 4)</td>
<td>37 (± 6)</td>
<td>75 (± 20)</td>
<td>N.S.</td>
<td>P &lt; 0.02</td>
</tr>
<tr>
<td>GDP</td>
<td>88 (± 8)</td>
<td>82 (± 10)</td>
<td>134 (± 17)</td>
<td>N.S.</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>G60D</td>
<td>43 (± 4)</td>
<td>43 (± 5)</td>
<td>77 (± 8)</td>
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</tr>
<tr>
<td>G120D</td>
<td>54 (± 5)</td>
<td>48 (± 6)</td>
<td>88 (± 14)</td>
<td>N.S.</td>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>IPD</td>
<td>49 (± 5)</td>
<td>52 (± 9)</td>
<td>146 (± 31)</td>
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</tr>
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<td>I60D</td>
<td>22 (± 3)</td>
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<td>38 (± 10)</td>
<td>N.S.</td>
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continued/.............
Table 25 (continued/....)

<table>
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<th></th>
<th>Control group</th>
<th>Early Treatment group</th>
<th>Late Treatment group</th>
<th>T test Control/Early Treatment group</th>
<th>T test Control/Early and Late Treatment group</th>
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<tr>
<td>I20D</td>
<td>32 (± 4)</td>
<td>32 (± 6)</td>
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<td>100 (± 19)</td>
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<td>H20E</td>
<td>44 (± 5)</td>
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<td>102 (± 28)</td>
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<td>CRT</td>
<td>1.5 (± 0.2)</td>
<td>1.3 (± 0.2)</td>
<td>1.2 (± 0.1)</td>
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<td>N.S.</td>
</tr>
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<td>1.9 (± 0.2)</td>
<td>1.6 (± 0.2)</td>
<td>2.7 (± 0.2)</td>
<td>N.S.</td>
<td>P &lt; 0.001</td>
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Clinical variables in the anorexia nervosa patients.

<table>
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<tr>
<th>Patient</th>
<th>Previous obesity</th>
<th>No history of obesity</th>
<th>Long duration illness</th>
<th>Short duration illness</th>
<th>Vomit</th>
<th>Bulimic</th>
<th>Purger</th>
<th>Overactive</th>
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</table>
Third Analysis - Stepwise multiple regression analysis.

The curve characteristics derived by the computer programme RSK 4 were subsequently examined by a stepwise multiple regression analysis to investigate the influence of some of the characteristics of the anorexia nervosa syndrome on the glucose tolerance and insulin response. The Statistical Package for the Social Sciences (SPSS) – version 5.01 was used (Nie, et al., 1975).

The characteristics treated as independent variables were previous obesity (PO), long duration of illness (DUR), bulimia (BUL), vomiting (VOM), purging (PUR) and over activity (ACT). A patient was classified as having been previously obese if at any time they had been more than 15% above their target weight. Patients who had suffered from anorexia nervosa for more than four years were classified as long duration of illness. The remaining clinical variables vomiting, activity, use of purgatives and bulimia did not lend themselves to such clear cut-off points. Here the population was simply divided into those where such behaviours had been a prominent and important component of weight control and those where it had been relatively unimportant. Details of these factors for each patient are given in Table 26.

The dependent variables analysed were as follows:–

basal glucose GB
basal insulin IB
peak glucose above origin GP
peak insulin above origin IP
peak glucose above basal GPD
peak insulin above basal IPD
mean glucose 0-60 minutes above origin G60
mean insulin 0-60 minutes above origin I60
mean glucose 0-60 minutes above basal G60D
mean insulin 0-60 minutes above basal I60D
mean glucose 0-120 minutes above origin \( G_{120} \)
mean insulin 0-120 minutes above origin \( I_{120} \)
mean glucose 0-120 minutes above basal \( G_{120D} \)
mean insulin 0-120 minutes above basal \( I_{120D} \)
mean glucose 60-120 minutes above basal \( G_{120E} \)
mean insulin 60-120 minutes above basal \( I_{120E} \)
The ratio \( G_{120E}/G_{60D} \) \( G_{RT} \)
The ratio \( I_{120E}/I_{60D} \) \( I_{RT} \)

Because it had already been observed that stage of treatment had a strong influence on glucose and insulin responses this independent variable was the first to be entered in the stepwise analysis of variance. Subsequently the other independent variables entered the analysis in order of their importance.

Results of the stepwise regression analysis

Basal levels

There was no significant difference between early and late treatment groups in basal levels of glucose. Previous obesity (partial \( r = -0.61, P < 0.05 \)) added 36% to the explained variance. Bulimia was also negatively correlated (partial, \( r = -0.57, P < 0.10 \)) and added a further 20% to the variance, this implies that at either stage of treatment patients who were previously obese or bulimic had lower basal glucose levels. Variation in basal insulin (IB) was not significantly related to, or explained by, the background variables.

Peak responses

Glucose

Peak glucose above basal (GPD) was significantly related to stage of treatment (\( r = 0.69, P < 0.05 \)). Bulimia was negatively related to GPD (partial, \( r = -0.66, P < 0.05 \))
and added a further 23% to the variance, that is bulimics had lower peak glucose levels. The regression was slightly improved by the inclusion of DUR (partial $r = 0.54$, $P < 0.10$) which added 8.5% to the explained variance. This implies that patients who had long standing anorexia nervosa tended to have higher peak glucose levels.

**Insulin**

Peak insulin above basal was significantly related to stage of treatment ($r = 0.75$, $P < 0.05$) and accounted for 56% of the variance. The regression was slightly but not significantly improved by the addition of VOM (partial $r = 0.46$, $P > 0.10$) which added 9.6% to the variance.

**Mean responses**

**Glucose**

All response measures (G60D, G120D, G120E) were significantly and positively related to stage of treatment ($P < 0.05$). The regression of G120D and G120E were significantly improved by BUL (partial $r = -0.71$, $P < 0.05$ and partial $r = -0.73$ and $P < 0.05$ respectively). A further improvement was caused by the addition of DUR (partial $r = 0.58$, $P < 0.10$ and partial $r = 0.66$, $P < 0.05$ respectively). BUL also made a non-significant improvement to the regression of G60D (partial $r = 0.51$, $P > 0.10$). This means that glucose tolerance tended to be more impaired in late treatment patients, and those with anorexia of long standing but less impaired in those patients who were bulimic.

**Insulin**

Stage of treatment was significantly related to I120D and I120E ($P < 0.05$) accounting for 40% and 43% of the respective variances, a similar but non-significant relationship held for I60D. The regressions were
improved, non-significantly by the addition of DUR, which was correlated negatively with insulin response, that is, long duration patients tended to have low insulin values.

**2 hour/1 hour ratios**

These indices were computed for glucose (GRT) and insulin (IRT) and should reflect delayed and sustained responses. For glucose no significant regression was obtained, but for insulin stage of treatment \( (r = 0.79, P < 0.05) \) accounted for 62.5% of the variance, and the regression was improved by the addition of VOM (partial \( r = 0.66, P < 0.05 \)) which accounted for a further 16% of the variance.

Thus sustained and late insulin responses were found in late treatment patients and in those who were known to vomit in order to induce weight loss.

The influence of the independent variables on glucose tolerance and insulin response is summarised below:

**Stage of treatment**

As observed by other methods of analysis the late treatment group showed more impaired glucose tolerance, delayed insulin peaks, high insulin levels and greater insulin resistance.

**Previous obesity**

Patients who were previously obese had lower basal glucose levels.
Bulimia

Bulimics had lower basal glucose levels and a lower peak glucose and less impaired glucose tolerance.

Duration of illness

Patients with long-standing anorexia nervosa had high peak glucose levels, impaired glucose tolerance and low insulin values.

Vomiting

Sustained and late insulin responses were found in patients who were known to vomit.

Activity and Purging

There was no influence of activity or purging on glucose tolerance or the insulin response to an oral glucose load.

DISCUSSION

Many factors have been said to influence blood glucose levels after an oral glucose load; previous carbohydrate intake (Wilkerson, et al., 1960; Conn, 1940; Irving, et al., 1954; Hales and Randle, 1963; Anderson and Herman, 1975) previous energy intake (Anderson, et al., 1972), the duration of periods of fasting prior to the test (Walsh, et al., 1973; Goldblatt, et al., 1932; Anderson and Herman, 1972) and the time of day (Jarrett, et al., 1969, 1970, 1972; Gøschke, et al., 1974). Moreover there is, in some individuals, a considerable amount of variation between tests carried out under the same conditions (O'Sullivan, et al., 1966; West, et al., 1964; McDonald, et al., 1965). Thus it is very important when performing glucose tolerance tests that the conditions are standardised for even then considerable inter-individual variation remains.
In the present study every attempt was made to standardise conditions during the test but certain variables remain. In anorexia nervosa there are different kinds of eating behaviour which may have different effects on glucose metabolism. Some anorectics avoid foods containing carbohydrate exclusively, others may follow alternate starve and stuff regimes, the periods of bulimina including the consumption of foods containing carbohydrate. Activity has also been shown to influence glucose metabolism (Bjorntorp, 1974) and some anorectics use exercise to control their body weight (Crisp, 1965; Dally, 1969).

The glucose tolerance was normal in the admission group who had eaten little carbohydrate and whose energy intake had been restricted for months before admission, moreover, the insulin curve followed the glucose curve closely. The early treatment group (which included the admission group) also showed normal glucose tolerance and pattern of insulin response. These findings are in sharp contrast to the findings in total starvation or even short term carbohydrate restriction. Hales and Randle (1963) put 5 normal healthy men on a diet containing only 50 g of carbohydrate for five days and found the low carbohydrate diet led to a reduction of glucose tolerance and a smaller rise of plasma insulin, however the fasting, 60 minute and 150 minute insulin values were higher on the low carbohydrate diet. The low carbohydrate diet also caused higher free fatty acid levels on fasting which stayed higher at 30 and 60 minutes after oral glucose but were the same at 150 minutes as on the high carbohydrate diet.

Wilkerson, et al. (1960) observed 15 women between 25 and 59 years old on varied carbohydrate and energy intakes. Diet one contained 250 g of carbohydrate and 2250 Kcals, diet two 300 g of carbohydrate and 2500 Kcals and diet three 50 g of carbohydrate and 1500 Kcals. The first two diets
were taken for three days but the third diet was taken for seven days. 100 g oral glucose tolerance tests were given on the day following each diet. None of the women met their criteria for diabetes on any of the experimental diets but the one hour blood glucose levels were significantly higher on the 50 g glucose diet. Wilkerson at the same time observed oral glucose tolerance in 42 men on varied carbohydrate intakes (400 g, 300 g, 100 g carbohydrate and high fat, 100 g carbohydrate and low fat, and 50 g carbohydrate respectively). None of the men met the criteria for diabetes on any of the diets and the only significant difference was on the one hour blood glucose values where those of the 100 g low fat, 100 g high fat and 50 g carbohydrate diets were higher than the 'usual' diet and the 300 g carbohydrate diet. The 100 g carbohydrate restorative diet for three days appeared to improve glucose tolerance but the 200 g carbohydrate restorative diet for three days did not give conclusive evidence of improved glucose tolerance. They concluded that the routine of high carbohydrate preparation for the oral glucose tolerance test has been exaggerated particularly when it is being used as a tool to diagnose diabetes.

It is worth noting that the 50 g carbohydrate diets for both the women and the men were low in energy content. It is recorded that the women lost weight. The weight loss and the energy restriction, which may have led to high free fatty acid levels in the blood, may have influenced the glucose tolerance.

In the present study, because the admission group had been on restricted carbohydrate and energy intakes one might have expected the glucose tolerance to have been poor, this was not so. The normal blood glucose levels that were observed could have been due to slow absorption or rapid utilisation or a combination of these factors. In one patient there
was a little evidence of poor glucose absorption, the glucose response was very flat but in the other four patients in the admission group this was not so, in all cases the 15 minute glucose value was higher than the premeal value indicating there was no delay in absorption. Flat glucose tolerance curves in anorexia nervosa have also been observed by Sheldon and Young (1938) and Ross (1938). It is interesting to note in the present study that the insulin peak followed closely the glucose peak suggesting that the insulin response to the glucose stimulus was normal in these patients.

The admission group were all under weight and most of them had probably very little adipose tissue. It has been observed that glucose tolerance is poor in the obese and improves with weight loss. It may be that impaired glucose tolerance, particularly that associated with insulin resistance, is only seen in persons with some adipose tissue, and that the insulin resistance is associated either with the size of the adipocytes or high circulating free fatty acid levels. The early studies on glucose tolerance after low carbohydrate intakes and restricted energy intakes do not include corresponding observations on plasma insulin, but the later ones suggest the poor glucose tolerance is due to altered tissue responsiveness to insulin (Tzagourhis and Skillman, 1970; Mahler and Szabo, 1970; McGraw, 1968) rather than insulin deficiency or insulin antagonism from growth hormone, glucagon, adrenalin or cortisol.

There is no suggestion in the present study that there is altered tissue responsiveness to insulin in the early treatment group, the glucose values are nearly back to the original values at 120 minutes.
Arguing teleologically it is not surprising that the glucose tolerance was normal in the admission group. Impaired glucose tolerance particularly with glycosuria would be 'wasteful' of the glucose presented to the tissues. If a person is emaciated then once the needs of the central nervous system for glucose have been met then any excess glucose should be rapidly transported to the 'hungry' lean body mass or adipose tissue in order that it should be conserved or used as an energy source for, as there is so little adipose tissue, the reserves of fatty acids will be low. There is evidence that the muscle tissue in emaciated anorectics is avid for glucose (Russell and Bruce, 1964; and Russell, 1965). They found the difference between capillary and venous glucose tolerance curves (Δ glucose) to be large. After one week's refeeding Δ glucose was smaller. On neither test occasion was impaired glucose tolerance observed indicating insulin insensitivity in those patients.

Acute starvation led to deterioration of oral glucose tolerance in normal people in the studies of Vance, et al. (1968) and Berson and Yalow (1965), and in many obese patients (Beck, et al., 1964; Unger, et al., 1963 and Schless and Duncan, 1966). A two week fast in obese patients with potassium supplementation was associated with a significant improvement in the oral glucose tolerance (Anderson, et al., 1969). Without potassium supplementation there was no significant change in the blood glucose levels 0 to 180 minutes during the oral glucose tolerance test, but at 180 minutes and 240 minutes the blood glucose levels were higher, and at 240 minutes significantly so. They found no correlation between plasma free fatty acids and glucose tolerance.
The phenomenon of diurnal variation of glucose tolerance in man has been observed by several workers, (Roberts, 1964; Bowen and Reeves, 1967; Jarret and Keen, 1969 and 1970; Jarret, Baker, Keen and Oakley, 1972) and the 'best' glucose tolerance is achieved in the morning tests. In the afternoon the blood sugar rise is greater and the insulin response is smaller. Jarret, et al. (1972) state that the major difference lies in the early part of the test, but this is not strictly true, the insulin response is most different early in the test but the glucose response is the same for the first 30 minutes then diverges from 60 to 120 minutes. It is suggested that the islet cells are responsible for the diurnal variation in oral glucose tolerance because diurnal variation in intravenous glucose tolerance has also been observed indicating that it is not the response of the islets to gut hormones that is responsible (Abrams, et al., 1968; Nemeth, et al., 1970). Ben-Dyke (1971) observed a diurnal variation in the nuclear size of rat islet cells as well as glucose tolerance.

In the present study for clinical reasons the tests were performed at mid-day. From other studies one would predict that such studies would give higher blood glucose levels and lower insulin levels than a morning test on the same patient. However, the control group provides a useful comparison with the anorectic groups and the different anorectic groups act as their own control. The control group did indeed show slightly impaired glucose tolerance and a rather sustained insulin response, but the most noticeable feature of the oral glucose tolerance tests and immuno-reactive insulin levels is that with re-feeding, as the anorectics gained weight, the glucose tolerance became progressively more impaired. This trend was seen in those individuals that were studied over a long period of time, as well as the comparison between early and late treatment groups. There was also a tendency for the peak insulin values to be higher and later as treatment progressed.
The insulin resistance may have been because part of the immuno-reactive insulin assayed was proinsulin and not insulin. Proinsulin is less effective in lowering blood glucose (Chance, Ellis and Bromer, 1968; Fineberg and Merrimee, 1970) and has a longer half-life than insulin but it cross-reacts with insulin antisera (Steiner, et al., 1967a, 1967b; Roth, et al. 1968). Although it is difficult to assay proinsulin in the presence of insulin, they can be separated by column chromatography (Gordon, et al., 1969) and subsequently assayed by radioimmunoassay. By such techniques it has been shown that the highest proportion of proinsulin is found in fasting blood samples from normal subjects, obese non-diabetics and diabetics (Melani, 1972). After oral glucose loading the actual levels of proinsulin increase but as a percentage of total insulin immuno-reactivity they fall.

Gordon, et al. (1969) found the proportion of 'big insulin' (identical with proinsulin) was higher at 15-30 minutes after oral glucose in obese subjects. Patients with tumours of the islets of Langerhans have consistently high proportions of proinsulin in the serum immuno-reactive insulin (Goldsmith, et al., 1969; Melani, 1972) but so far there have been no other cases reported where apparent insulin resistance is due to a large proportion of proinsulin in the immuno-reactive insulin. Becker, Murray, Hansen and Pimstone (1973) investigated circulating 'big' insulin in protein-energy malnutrition and found there was no excessive secretion of big insulin, and so it could not account for the sustained insulin response sometimes found in such children (Becker, et al., 1972). It is thus unlikely, but possible, that the insulin resistance of patients in the present study is due to large quantities of proinsulin.

The insulin resistance may have been of the type seen in maturity onset diabetes and obesity, where insulin resistance may be related to the nature of the receptor sites on cell membranes. Abnormal tissue
responsiveness to insulin may be due to

a) a decreased number of binding sites
b) a decreased 'affinity' of binding sites for insulin
c) an increased rate of dissociation of insulin from the binding sites, or
d) abnormality in transmission of the 'signal' arising from the insulin–receptor interaction.

In rodents both genetic and acquired forms of obesity are characterised by decreased insulin binding to receptors not only on adipose tissue but also liver, muscle and lymphoid tissue, (Olefsky and Reaven, 1975; Kahn, et al., 1973; Freychet, et al., 1974; Soll, et al., 1974). In human obesity weight reduction through dietary caloric restriction results not only in restoration of insulin sensitivity of adipose tissue and thus improved glucose tolerance but also improvement of binding of insulin to circulating monocytes (Archer, et al., 1975).

This is not altogether surprising in view of the very recent finding (Schramm, et al., 1977) that receptors can be transferred from one cell to another by cell fusion techniques and linked to the adenyl cyclase of another cell and that all hormone receptors which couple to adenyl cyclase probably have a common chemical structure differing only in the confined area which binds the specific hormone.

In their early work Salans, et al. (1968) suggest that 'stuffing' the adipose cell with lipid may distort the cell membrane resulting in a change in the receptor sites or in some membrane constituent such as adenyl cyclase which may be involved in the intracellular action of insulin. However, later work on insulin receptors of other tissues (Archer, et al., 1975) suggests that it is not necessary to have a large amount of lipid
within the cell in order for insulin binding or sensitivity to be impaired. Jacobsson, et al. (1976) also confirmed this in their study of large and small human fat cells, where they showed that cellular sensitivity was similar in cells of different size but that the capacity to respond was greater in large cells. They also noted that the concentration of insulin needed to obtain an antilipolytic effect was far below that needed to stimulate glucose incorporation and interpreted this in terms of binding of insulin to receptors with different affinity.

Whatever the mechanism of the insulin resistance seen in patients of the present study it appears likely that it is the food restriction rather than the reduced adipocyte size which is important in altering insulin binding to receptor sites, and the comparative overfeeding which precipitates the insulin resistance observed. The anorexia nervosa patients, even after refeeding, were not obese and although estimations of adipocyte size were not made it is unlikely that their adipocytes were larger than those in Archer's study (1975) where extremely obese subjects who had lost relatively small amounts of weight showed improvement of glucose tolerance. Thus it appears that normal people have normal insulin binding and sensitivity on high food intakes, that the obese have defective insulin binding and glucose intolerance on normal and high food intakes but that caloric restriction can restore normal binding and glucose tolerance, whereas anorectics have normal glucose tolerance when underfed and emaciated, but impaired glucose tolerance when refeed to normal weight on food intakes that would not be excessive for normal individuals.

There is confusion in the literature about the changes in carbohydrate metabolism associated with anorexia nervosa.
Landon, et al. (1966) reported increased sensitivity to intravenous insulin assessed on the basis of the lowest levels of blood glucose reached and the recovery index but Vigersky, et al. (1976) found their anorectic patients were slightly less insulin sensitive than normal subjects when assessed on the nadir of plasma sugar as a percentage of basal sugar. However, both groups agree that the rate of return of plasma sugar was slow. Vigersky showed the severity of this impairment was significantly correlated with the severity of weight loss - the most emaciated patients having the most persistent hypoglycaemia. In Mecklenberg's (1974) observations on five anorectic patients two had relatively normal responses to intravenously administered insulin, three however had a greater than 50% reduction in blood sugar and persistent hypoglycaemia.

Thus it may be concluded that emaciated anorectics tend to be more sensitive to insulin. Insulin is sometimes used in the treatment of anorexia nervosa but it should be used with care. In one case where insulin was given and the patient did not have breakfast afterwards, the coma which ensued caused irreversible brain damage and £93,000 damages were awarded (O'Brien, 1976). The persistent hypoglycaemia observed at the end of the insulin tolerance test may be due to decreased insulin degradation, glucagon insufficiency or defects in the major gluconeogenic pathways. The finding that plasma glycerol levels were persistently higher in emaciated anorectics than in re-fed patients (Kanis, et al., 1974) even though plasma free fatty acid levels were much the same before and after treatment, suggests that there is some liver defect in converting this potential source of glucose.

These are two conflicting reports of the insulin response to intravenously administered glucose in anorexia nervosa patients. Stephan.
et al. (1972) found a normal response in eight emaciated anorectics who had not been refed whereas Crisp, et al. (1967) found impaired glucose tolerance and insulin resistance in newly admitted anorectics which remained after 8-10 weeks treatment.

Crisp gave 25 g of glucose to both controls and anorectics whereas Stephan dosed according to body weight (0.33 g/kg). The higher blood sugar rise that Crisp observed in his anorectics at 5 minutes following intravenous glucose probably reflects the smaller glucose pool of the emaciated patients, and the slightly higher insulin levels are the response to the higher blood glucose in these patients. However, after refeeding Crisp's anorectics had lower blood glucose levels although still higher than the control group, but higher insulin levels particularly at 30, 45 and 60 minutes after the glucose load, indicating that these anorexia nervosa patients after refeeding and weight gain were more insulin resistant. There is some indication of a similar insulin resistance developing in one other anorectic patient tested with oral glucose before and after weight gain (Frankel and Jenkins, 1975).

Kanis, et al. (1974) suggested that high fasting growth hormone levels were responsible for the insulin resistance they observed in five refed anorectics. However, Harrower, et al. (1977) have shown that lowering the growth hormone levels in similar patients by bromocriptine treatment did not change glucose or insulin levels during oral glucose tolerance tests. In fact after two weeks treatment with bromocriptine there is even some evidence of higher insulin levels. So it is unlikely that high growth hormone levels contribute to the insulin resistance seen in anorexia nervosa patients after weight gain on high food intakes.
'Since intestinal hormones form a fundamental part of the regulation of insulin release it is essential to consider them in all pathological conditions involving an abnormality of insulin release', (Marks and Turner, 1977). Anorexia nervosa is just such a condition. Three gastrointestinal hormones may be responsible for the augmented insulin response following oral glucose, gastric inhibitory polypeptide (GIP), intestinal insulin-releasing polypeptide (IPP) and gut glucagon-like immunoreactivity (enteroglucagon).

There is some evidence that intestinal hormones are involved in the hyperinsulinaemia of obesity and maturity onset diabetes. In many ways the insulin resistance of the present study is similar to that observed in these conditions.

The number of gastric inhibitory polypeptide and glucagon-like immunoreactivity - secreting cells has been shown to be increased in the intestine of the obese-hyperglycaemic mouse which also has gross β cell hyperplasia and hyperinsulinism. Obese human subjects have increased fasting GIP levels and show an exaggerated rise in plasma IPP after glucose by mouth. It has been suggested, by Marks and Turner (1977) in their review of the role of intestinal hormones in insulin release, that these hormones not only control insulin release acutely, but that they may also 'prime' the β cells in some way to render them more responsive to the stimulus of glucose. Insulin release may be considered in two phases, in the first phase the gut hormones act directly and stimulate insulin release, in the second phase the gut hormones stimulate insulin release by making the β cells more responsive to glucose. The delayed insulin response observed in maturity onset diabetes and in the refed anorectics of the present study may be because the glucose
threshold at which the gut hormones make the β cells responsive to glucose is for some reason higher in such patients. The gut hormone levels would also stay relatively higher because it would be longer before the feedback mechanism of high insulin levels depressing gut hormone levels comes into force. The threshold blood glucose concentration at which blood glucose stimulates insulin release is much lower in the presence of IRP or GIP than in their absence and so an alternative explanation would be that there is a low gut hormone response in the refeed anorectics, which means that blood glucose levels have to rise more before they stimulate insulin release. The influence of gut hormones on insulin release in anorexia nervosa can only be resolved by further experiments.

The impact of bulimia, vomiting, previous obesity, duration of illness, activity and purging on glucose tolerance in anorexia nervosa have not been investigated by other workers. The main findings of the multiple regression analysis apart from the strong influence of stage of treatment on glucose tolerance and insulin response were that bulimics had low basal glucose levels and less impaired glucose tolerance, that previously obese patients had lower basal glucose levels, that patients with anorexia nervosa of long standing had impaired glucose tolerance and the surprising finding that delayed and sustained insulin responses were found in patients who vomited habitually. The better glucose tolerance found in bulimic patients may be because the restriction of energy intake is not generally so severe in these patients and they also consume considerable quantities of carbohydrate during bulimic episodes.

It appears that long term carbohydrate avoidance and energy restriction predisposes to impaired glucose tolerance on refeeding. Affluent societies generally consume relatively little carbohydrate. In
the United Kingdom there has been a general trend of decreasing carbohydrate consumption and increasing fat consumption which was lead by the higher social classes. Diabetes mellitus is less common in some countries with a high carbohydrate intake (West, et al., 1966) and it has also been shown that diabetic patients consuming a diet high in carbohydrate (64% of total calories) and low in fat (20% of total calories) maintained good to excellent regulation without an increase in insulin requirements (Stone, et al., 1963). It appears from the epidemiological evidence as well as from the present experimental work with anorexia nervosa patients that low carbohydrate diets should not be recommended because they may predispose to diabetes.

Anorexia nervosa is said to be more common in the upper social classes, these are also the groups with a low carbohydrate consumption. As the lower social classes in the United Kingdom gradually reduce their carbohydrate consumption it will be interesting to see if the prevalence of anorexia nervosa increases. It may be that a restricted carbohydrate consumption not only predisposes to diabetes mellities but also to anorexia nervosa.
CHAPTER FIVE

PLASMA GLUCAGON LEVELS DURING RECOVERY FROM ANOREXIA NERVOSA
CHAPTER 5

PLASMA GLUCAGON LEVELS DURING RECOVERY FROM ANOREXIA NERVOSA

Introduction

Results

- N-terminal glucagon
- C-terminal glucagon
- 'Gut' glucagon

Discussion
Figure 35: Plasma N terminal glucagon levels after 100 g glucose by mouth in 15 anorexia nervosa patients (26 tests) and 6 control subjects (6 tests).
INTRODUCTION

Plasma glucagon levels were measured using Buchanan’s antibodies YY57 and YY89. Full details of the glucagon assay used in the present study are given in Appendix A. Antibody YY57 reacts with N-terminal fragments of pancreatic glucagon, total chicken glucagon and large molecular weight forms of gut glucagon-like immunoreactivity (GLI). Glucagon measured with this antibody is subsequently called N-terminal glucagon. Antibody YY89 reacts with C-terminal fragments of pancreatic glucagon, but reacts poorly with total chicken glucagon and not at all with large gut GLI. Glucagon measured with this antibody is subsequently called C-terminal glucagon.

RESULTS

N-Terminal Glucagon levels

There were no statistically significant differences between N-terminal glucagon levels in the anorectic group and the control group. One of the control group, C5, had consistently higher N-terminal glucagon levels than the other control subjects throughout the test and one late treatment anorectic, (210), had very high levels throughout the test, 340-2000 pg/ml. The results including both of these people are shown in Figure 35. There was no significant difference between the anorectic groups at different stages of treatment, and no clear pattern of response to the glucose meal in either the control or anorectic groups.

C-Terminal Glucagon levels

There were no statistically significant differences between C-terminal glucagon levels in the anorectic group and the control group (Figure 36). There was no
Figure 36: Plasma C terminal glucagon levels before and after 100 g glucose by mouth in 15 anorexia nervosa patients (26 tests) and 6 control subjects (6 tests).
Figure 37: Plasma C terminal glucagon levels before and after 100 g glucose by mouth in 6 early treatment anorexia nervosa patients (8 tests) and 8 late treatment anorexia nervosa patients (10 tests).
Figure 38: Plasma C terminal glucagon levels before and after 100 g glucose by mouth in 12 anorexia nervosa patients with one or more tests with at least one value > 160 pg/ml (16 tests) and six control subjects (six tests).
significant difference between anorectics at different stages of treatment, (Figure 37). The pattern of response to oral glucose is similar in the anorectic groups with a post glucose decrease in C-terminal glucagon reaching a minimum at about 45 minutes and then an increase to pre-glucose values from 90 to 150 minutes. There is little evidence of a similar pattern in the control group.

12 of the 15 anorexia nervosa patients had one or more test where at least one glucagon value exceeded 160 pg/ml (total number of tests 16). The results of these tests are given in Figure 38.

'Gut' Glucagon

Traditionally values of 'gut' glucagon have been derived by subtraction of the glucagon-like immunoreactivity (GLI) measured using a pancreospecific antibody from the GLI value determined using an antibody that cross-reacts with glucagon from the pancreas and gut extracts. Buchanan and his group had previously assessed the antibodies used in the present study as pancreospecific YY89 and cross-reacting YY57. If the YY89 values are subtracted from the YY57 values in this study in the anorectic group (26 tests, 15 patients) 6 tests gave all positive values; 4 tests gave all negative values and 16 tests gave mixed positive and negative values throughout the test. In the control group (6 tests, 6 subjects) 2 subjects gave all positive values; one subject gave all negative values and 3 subjects gave mixed positive and negative values. In a few tests only one negative value was recorded, if these are added to the positive group, nine anorectic tests and four control tests gave positive values. Figure 39 shows the so-called gut glucagon response to oral glucose in the 6 anorectics (9 tests) and 4 control subjects who gave positive values. The very high peak in the anorectic group is as the result of one very high value 1960 pg/ml (patient
Figure 38: Plasma 'gut' glucagon after 100 g glucose by mouth in six anorexia nervosa patients (9 tests) and four control subjects (4 tests).
210 at 45 minutes after oral glucose). This value may not easily be rejected as erroneous because it is in scale with the other values for this patient during the test.

**DISCUSSION**

Glucagon was first recognized as a mobiliser of glucose in 1923 by Murlin and his colleagues, since then it has been isolated and purified, the molecular structure has been determined and confirmed by synthesis and other metabolic and physiological functions of glucagon, such as its role in lipid and amino acid metabolism, have been demonstrated (Murlin, et al., 1923; Staub, et al., 1953, 1955; Lefebvre and Marliss, et al., 1972).

The pathophysiology of glucagon has also been investigated and attempts have been made to determine its importance in diabetes mellitus, obesity, starvation, hyperparathyroidism, chronic renal failure, trauma and severe burns (Dobbs, et al., 1975; Sakurai, et al., 1975; Unger, et al., 1975; Kalkhoff, et al., 1973; Wise, et al., 1973; Muller, et al., 1971; Aguilar-Parada, et al., 1969; Lawrence, 1972; Kuku, et al., 1976; Marliss, et al., 1972; Unger and Orci, 1976; Wilmore, et al., 1974).

Two extensive reviews of the physiology and pathophysiology of glucagon have been published, in 1972, a book with many contributors, 'Glucagon, molecular physiology, clinical and therapeutic implications', edited by Unger and Lefebvre, and in 1976 a review by Unger and Orci.

Many problems face investigators interested in glucagon, not least among them being the methods available for measuring glucagon in tissues, tissue extracts and body fluids. Bioassays for glucagon are available but none have all the features of specificity, sensitivity, accuracy and simplicity which are desirable. *In vivo* assays (Staub and Behrens, 1954) and liver slices (Vuylsteke and De Duve, 1957) although relatively simple have poor
specificity. Subcellular preparations (Makman and Sutherland, 1964) and perfused livers (Sokal, 1970) have greater specificity but more technical skill is required and in the case of perfused liver the cost of each assay is high. Thus bioassays are more commonly used to determine whether a material has the biological activity of pancreatic glucagon and radioimmunoassays are used for routine quantitative analysis. However, radioimmunoassays have their own problems of specificity. Glucagon is secreted not only by the A2 cells in the islets of Langerhans, but also by A-like cells in gut tissues, such cells have been identified in the duodenum of man. It is not yet clear in what ways glucagon from these two sources differs either in form or in function.

The early glucagon assays used non-specific antibodies which cross-reacted with gut extracts (Unger, et al., 1963; Samols, et al., 1965; Buchanan, et al., 1967) but later, assays with so called pancreospecific antibodies were developed (Aguilar-Parada, et al., 1969; Samols, et al., 1969; Heding, 1971). Unger's pancreospecific antibody 30K and Buchanan's pancreospecific antibody YY89 (used in the present study) both react with the C-terminal of glucagon but they differ in specificity because 30K reacts with large immunoreactive glucagon (IRG) and YY89 does not. Both Unger's 'cross-reacting' antibody 78J and Buchanan's 'cross-reacting' antibody YY57 react with the N-terminal of glucagon. IRG in plasma and in pancreatic tissue is heterogenous, usually three fractions can be identified, the first with a molecular weight in excess of 40,000 macroglucagon; the second with a molecular weight of approximately 9,000 (called large glucagon or proglucagon) and a third which corresponds with the labelled glucagon marker and a molecular weight of 3,485 (true glucagon). Moreover, there is evidence that extrapancreatic glucagon can be separated into very similar components (Valverde, et al., 1975; Sasaki, et al., 1975). It may thus be concluded
that radioimmunoassays for glucagon at present probably cannot differentiate between true glucagon (molecular weight 3485) and other high molecular weight forms with lesser biological activity nor can one be certain that they differentiate between glucagon of pancreatic origin and gut glucagon.

In fact there may be no physical or chemical difference between the glucagon from different source, just a difference in the stimuli that cause secretion. It would appear that the N-terminal of glucagon is usually exposed and can react with antibodies specific for that terminal (cross-reacting antibodies) but that the C-terminal is not always so readily available (pancreospecific antibodies). Biological activity appears to be linked with the availability of the C-terminal to react with antibodies.

As recommended by Kuku, et al. (1976) I will interpret the significance of circulating IRG levels of the present study with caution.

I could find no other reports of glucagon levels in patients with anorexia nervosa. It would appear from the present study that C-terminal glucagon levels are relatively high in some anorexia nervosa patients. The patients refeeding and weight gain did not apparently influence these glucagon levels.

Hyperglucagonaemia has been found in cell tumours, acromegaly, pheochromacytoma, diabetes mellitus, hyperparathyroidism, polyglandular syndrome, obesity, starvation ketosis, pancreatitis, chronic renal failure and trauma (Lawrence, 1972; Unger and Orci, 1976). Glucagon levels are also raised in healthy individuals who are starved or on restricted carbohydrate intakes, but in these cases normal food and carbohydrate intakes quickly lower the glucagon levels to normal values (Marliss, et al., 1970; Muller, et al., 1971).
Hyperglucagonaemia detected by radioimmunoassay may be the result of any of the glucagon fractions in plasma. After stimulation tests with arginine the rise in glucagon is largely due to an increase in true glucagon (3,485 molecular weight), whereas the hyperglucagonaemia of chronic renal failure is largely due to an increase in IRG of approximately 9,000 molecular weight (Valverde, et al., 1975; Kuku, et al., 1976).

In the present study it is likely that the high C-terminal glucagon levels observed in some of the anorexia nervosa patients correspond with high levels of true glucagon as the antibody used, YY89, does not react with large gut glucagon. The high glucagon levels in early treatment are not surprising, for glucagon levels are known to be elevated during starvation or restricted carbohydrate intake, but the failure of refeeding to restore these glucagon levels to normal suggests some abnormality of glucagon secretion. The glucoreceptor, glucose-sensing, and glucagon-suppressing mechanisms remain to be elucidated; a direct action of insulin on the A cell and an indirect action of insulin involving either transport or metabolism of glucose have been postulated (Samols and Marks, 1972; Unger and Orci, 1976).

Although newly admitted anorectics have high glucagon levels it is not effective in promoting gluconeogenesis from glycerol (Kanis, et al, 1974). Physiologic glucagon resistance has been reported in the generating fetal liver and the regenerating liver of partially hepatectomized adult rat (Unger and Orci, 1976) it appears that anorexia nervosa may provide another example of glucagon resistance.

The observations on N-terminal glucagon and 'gut' glucagon levels in the present study demonstrate the methodological difficulties associated with glucagon assays. Buchanan (personal communication) has similar results...
when trying to assess 'gut' glucagon levels. He suggests that present techniques are not adequate to measure the various species of glucagon. The assays for C-terminal glucagon, however, appear to be a fairly reliable index of 'true' glucagon.
CHAPTER SIX

SOME METABOLIC ASPECTS OF DIETARY THERMOGENESIS
Metabolic rate is raised after eating – this is not surprising because food is absorbed as simple chemical constituents, glucose, fatty acids, amino acids and subsequently after transportation to liver and peripheral tissues is built up into more complex molecules – glycogen, fat and protein. These syntheses use ATP which must be derived from oxidation of glucose or fatty acids. Thus the storage of future energy supplies and the synthesis of protein is energy demanding, and as the substrate supply is greatest post prandially, one can predict a raised metabolic rate at that time.

The heat production possible from energy deposition was summarised by Millward, et al. (1976) and is given in Table 27. The least costly process is the conversion of dietary fat via free fatty acids to tissue fat (0.01 kJ heat/kJ fat deposited). The deposition of fat from dietary carbohydrate on protein produces 0.15 kJ heat/kJ of fat deposited. The most inefficient process is probably the deposition of dietary protein as fat. If this occurs via the intermediate formation of glucose and ketones in the liver then 0.31 kJ of heat would be produced for each kJ of fat deposited. The extent to which this occurs is not known, but it is possible that most excess amino acids are directly oxidised. The oxidation of amino acids is also a poorly phosphorylating pathway and the heat production associated with a given amount of ATP production would be about 10% higher than from other sources.
Table 27

Heat production from energy deposition\(^1\)
(from Millward, et al., 1976)

<table>
<thead>
<tr>
<th>Heat</th>
<th>kJ/kJ deposited</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat into fat(^2)</td>
<td>0.01</td>
</tr>
<tr>
<td>Triglyceride → free fatty acids → Triglyceride</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate into fat(^3)</td>
<td>0.15</td>
</tr>
<tr>
<td>14 glucose + 12 O(_2) → Tripalmitylglycerate + 33 CO(_2) + 21 ATP</td>
<td></td>
</tr>
<tr>
<td>Protein into fat(^4)</td>
<td>0.31</td>
</tr>
<tr>
<td>21 Amino acids + 48.3 O(_2) → Tripalmitylglycerate + 36 CO(_2) + 14.4 Urea</td>
<td></td>
</tr>
<tr>
<td>Protein into protein</td>
<td>0.15</td>
</tr>
<tr>
<td>1 Amino acid + 5 ATP → 1 peptide</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Calculations based on molecular wts of 806, 162 and 110 and heat contents of 39.6, 17.5 and 18.1 kJ/g for triglyceride, carbohydrate and protein. ATP is assumed to be formed from dietary carbohydrate at a rate of 36 mol/mol glucose.

\(^2\)Assuming a requirement of 6 ATP per mole triglyceride.

\(^3\)Assuming conversion as described in McGilvery (1970).

\(^4\)Assuming conversion via glucose and ketones as described in McGilvery (1970).
The post prandial increase in metabolic rate is probably best explained in terms of the heat production from conversion of fat into fat, carbohydrate into fat and protein into protein. It is obvious from the figures given in Table 27 that the quantity of substrate provided by the meal will influence the size of the thermic effect much more than the nutrient composition of the meal. This is in agreement with the experimental evidence discussed more fully in the first chapter of this thesis (Bradfield, 1973; Miller, et al., 1967; Kriss, 1941).

Two factors have been said to increase the thermic effect of a meal, exercise and growth. In the present study although the thermic effect of a meal was shown to be present in exercising subjects there was no conclusive evidence that exercise potentiated the thermic effect. It is difficult to explain any exercise potentiation of the thermic effect of a meal in biochemical terms. During post prandial exercise one would predict that a greater proportion of the absorbed nutrients, particularly carbohydrate, would be directly oxidised and thus the amount of fat synthesis from carbohydrate reduced. Consequently there would be less heat production from the synthetic processes. At rest approximately 15% of a 100 g oral glucose load is available to the insulin dependent peripheral tissues (Felig, et al., 1975b). Most of the glucose (55%) is retained by the liver and used for glycogen synthesis, triglyceride formation or glycolysis. During exercise, however, the liver is a net producer of glucose (Felig, et al., 1975a) and probably does not retain such a high proportion of the oral glucose load. Although I could find no direct experimental evidence that this is so. The lower energy cost of post prandial exercise seen in one subject of the present study can be explained in terms of a larger proportion of the nutrients from the meal being directly oxidised, consequently the energy demand for synthesis of triglycerides will be reduced.
The large thermic effects of meals during recovery growth as observed by Brooke and Ashworth (1972) in malnourished infants and in the anorectics of the present study may be explained in terms of a greater proportion of any nutrients from the meal going into synthetic processes rather than direct oxidation. The insulin sensitivity of the newly admitted anorectic probably reflects the predominately anabolic and conservative metabolic posture of these patients.

In Chapter One I questioned briefly whether the thermic effect of meals was an integral part of luxusconsumption and the regulatory process which controls energy balance. Although the phenomena are obviously related I do not think there is a close relationship. The size of the thermic effect of a meal is most closely related to the size of the meal. There may be differences in the immediate metabolic fate of the nutrients from a meal in obese and normal weight individuals, and it may be that a greater proportion of the nutrients are directly oxidised in the obese, but the differences in the size of the thermic effect of a meal are not usually large enough to explain the increased overall metabolic rate in overfeeding. The thermic effect of a meal is the metabolic response to a sudden increase in the substrate supply whereas luxusconsumption and the reduced metabolic rate on underfeeding involve changes in turnover of metabolites.

It has been suggested that changes in protein turnover may be responsible for the high energy requirement of growth and the low energy requirement when food intake is reduced. Millward, et al. (1976) suggest that changes in protein turnover are not the source of all the extra heat associated with growth, and that although protein turnover is reduced in obese man on a restricted food intake the change in protein turnover falls in parallel with other heat producing pathways.
Luxusconsumption in man and rats has been shown on diets that are very low in protein (Miller, et al., 1967; Stirling and Stock, 1968), moreover Sims (1976), when feeding extra carbohydrate, found human subjects gained less weight than those fed on equivalent caloric excess but with less carbohydrate and more fat. It is most likely that these high carbohydrate regimes altered turnover of nutrients other than protein, and so increases in protein turnover can not be responsible for luxusconsumption in these cases. Substrate cycling can occur either within tissues or between tissues. The energy cost of transport between tissues would increase the possible heat production from substrate cycling.

Subtle changes in thyroid hormone metabolism have been shown in overfeeding and protein-energy malnutrition. Thyroxine (T₄) is peripherally altered to T₃ and 3,3',5 triiodothyronine – reverse T₃. Reverse T₃ is less calorigenic than T₃. In Sims, subjects who were overfed on carbohydrate T₃ levels rose and reverse T₃ declined (Sims, 1976). The opposite effects were reported in protein energy malnutrition (Chopra, et al., 1975). One of the mechanisms by which thyroid hormones exert their influence is by increasing the activity of the sodium pump (Edelman, et al., 1974) but I know of no evidence that ionic flux is altered in luxusconsumption.

The inefficient pathways associated with luxusconsumption may arise because the large substrate supply either directly from absorbed nutrients or indirectly from increased turnover of nutrients swamps the efficient pathways of ATP production. An example of more wasteful pathways as a result of increased supplies of substrate is lipogenesis. Lipogenesis is limited by the supply of reduced NADP. Reduced NADP is generated by the hexose monophosphate pathway. If lipogenesis were to proceed at rates only limited by the supply of substrate the demands on the hexosemonophosphate pathway would be so great that less glucose would be

See page 188
routed through the glycolytic chain and the Kreb's cycle, this would lead to restricted ATP production. Cellular ATP levels appear to be maintained at all costs, and thus, when the substrate supply for lipogenesis is large, the extra reduced NADP required is generated by the malate shunt. The malate shunt is less efficient and generates more heat. In this case homeostatic mechanisms for maintaining cellular ATP levels together with an increased supply of nutrients results in less efficient lipogenesis.

What is not clear, and needs to be investigated, is the extent to which wasteful cycling and less efficient pathways are controlled in order to regulate energy balance and body weight. It seems that man is well adapted to use food efficiently. The ability to do so is advantageous when food supplies are uncertain. Whether man has also developed an ability to luxusconsume in order to maintain the 'advantages' of a slim physique in the presence of an abundant supply of food needs to be confirmed. Luxusconsumption may be a fortuitous consequence of increased substrate supply rather than a regulatory mechanism for maintaining energy balance and body fat content.

In the present study the patients who had no history of previous obesity not only adapted better to their self-imposed food restriction by maintaining a lower metabolic rate/kg body weight but also adapted better to increased food supplies by increasing metabolic rate. This does not necessarily mean that body weight is regulated in these individuals and that regulation of body weight is faulty in those patients with a previous history of obesity, it may just mean that their body weight reaches different equilibrium points on restricted or excessive food intakes. A regulatory mechanism implies a detector of body energy stores and a feedback mechanism whereby these stores can be controlled.
In practice, individuals vary in their tendency to fatness. When the biochemical and metabolic mechanisms for this are fully understood appropriate treatments for obesity will be easier to devise. The DHSS/MRC report (1976) 'Research on Obesity', states 'obesity is neither one condition nor one disease'. Care must be taken when the biochemical mechanisms are investigated not to ignore metabolic differences just because they are found in a few cases. It may be that the metabolic difference observed in those subjects caused their obesity, and that other metabolic changes cause obesity in other cases. There are very many alternative pathways for wasteful cycling, and poorly phosphorylating pathways.

Note: Since this thesis was completed York et al (1978) have found the ATPase involved in the transport of sodium into cells is deficient in genetically obese mice. This enables the obese mice to use their food more efficiently.
GLUCAGON ASSAY

The glucagon assays were carried out by the author of this thesis in the Department of Medicine, Queen's University of Belfast, under the direction of Professor Keith Buchanan using the assay procedures that he had developed.

Extraction of plasma

Plasma samples were extracted prior to the assay of glucagon. The extraction was carried out at ice temperature. All tubes were kept in racks in trays of crushed ice in water. Duplicate 0.5 ml aliquots of plasma were taken, 0.8 ml 96% alcohol added to each and mixed vigorously by hand for a few seconds. The extraction mixture was centrifuged at 4°C and 2500 r.p.m. for 30 minutes. The supernatants from both aliquots were then decanted into the same wide necked glass evaporating vessel (2 dram size with stoppers from Johnsen and Jorgensen Ltd., Herringham Road, Charlton, London, S.E.7.). They were evaporated to dryness in a dessicator attached to a vacuum line. The extracted samples were stoppered and stored at 4°C.

Glucagon assay

Buffer

0.04 M phosphate buffer, pH 7.4 was made as follows:

For 25 litres

\[ \begin{align*}
115 \text{ g } & \text{Na}_2\text{HPO}_4 \\
29.87 \text{ g } & \text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O} \\
5 \text{ g } & \text{thiomersalate}
\end{align*} \]

Make up to 25 litres with distilled de-ionised water.

Bring pH to 7.4 with NaOH (approximately 2 N).
This buffer can be stored in bulk at 4°C for 2-3 months. The buffer was prepared with 2% horse serum (Burroughs Wellcome No.5) for all procedures in the assay. Aliquots from a freshly opened bottle of horse serum were stored frozen because the use of serum from opened bottles, even those stored at 4°C, can result in bacterial contamination and gross destruction in the assay. These aliquots were thawed and added to the 0.04 M phosphate buffer just before the assay was set up.

Standards

The standards were prepared from an MRC standard in acid alcohol (3:1 v/v 0.7 M HCl; ethanol). The standard was diluted in acid alcohol to 100 µg/ml concentrated stock, aliquotted in approximately 0.5 ml amounts in LP3 tubes and stored in the deep-freeze at -20°C. Every 2-4 months a working stock standard was prepared from this concentrated stock in acid alcohol as follows:

0.1 ml 100 µg/ml concentrated stock to 10 ml acid alcohol = 1000 ng/ml. This was aliquotted in approximately 0.5 ml amounts in LP3 tubes and stored at -20°C. On the day of the assay daily working standards were prepared from the 1000 ng/ml working stock, strict ice temperatures were observed.

10 µl of 1000 ng/ml working stock to 4 ml zero plasma extract = 2500 pg/ml daily working standard.

For the standard curve 2 ml doubling dilutions were made in zero plasma extract to give 2500, 1250, 625, 313, 156, 78, 39 pg/ml. The same volumetric pipette was used throughout. It was rinsed twice at each stage in each solution, not distilled water. The standards and concentrated and working stock aliquots were discarded after use.
Charcoaled plasma

Human heparinised plasma was added to 2 g% charcoal and mixed thoroughly, centrifuged and the supernatant plasma decanted. The plasma was mixed again with 2 g% charcoal, centrifuged and the supernatant filtered until the filter papers were free of charcoal (three times with Sartorius filter 47 mm 5 M 13400 and three times with 50 mm 5 M 11306).

Zero plasma extracts

0.5 ml charcoaled plasma + 0.8 ml 96% alcohol were mixed vigorously, centrifuged, and the supernatant extract decanted into a glass evaporating vessel. 10 extracts were pooled and evaporated to dryness in a dessicator under negative pressure. The pooled extract was labelled with a serial number and 5 ml.

Pancreatic recovery tubes

20 μl of 1000 ng/ml glucagon working stock standard were made up to 25 ml in a volumetric flask with ice cold zero plasma and mixed carefully to avoid frothing.

0.5 ml aliquots of pancreatic recovery plasma were extracted with 0.8 ml of alcohol as above (p.189).

Two extracts were pooled, labelled PR and with a serial number and 1 ml.

Gut recovery tubes

50 μl of undiluted gut extract were made up to 25 ml in a volumetric flask with ice cold zero plasma, 0.5 ml samples were extracted. The extracts were pooled in pairs labelled GR and with a serial number and 1 ml.
The gut extract was prepared by the method of Murphy Buchanan and Elmore (1973).

**Antibodies**

Radioimmunoassays for plasma glucagon use antisera that are specific for pancreatic glucagon to measure pancreatic glucagon (Heding, 1971) and antisera which cross-react with gut extracts as well as pancreatic glucagon to measure total glucagon-like immunoreactivity (GLI). By subtraction of the pancreatic glucagon value from the cross-reacting GLI value an estimate of the gut GLI can be made.

Buchanan's group have defined the specificities of two antibodies referred to as YY89 and YY57. YY89 is pancreatic specific and YY57 cross-reacts with gut GLI fragments as well as pancreatic glucagon.

In 1973, Flanagan, Buchanan and Murphy reported they had investigated these antisera further using chicken glucagon, fragments of pork glucagon and highly purified fractions of gut glucagon. They found that antibody YY57 reacts with N-terminal fragments of pancreatic glucagon, total chicken glucagon and large molecular weight forms of gut GLI. Antibody YY89 reacts with C-terminal fragments of pancreatic glucagon, but reacts poorly with total chicken glucagon and not at all with large gut GLI.

When crude gut GLI was added to serum for recovery experiments some material was found which reacted with YY89 antisera. YY89 had previously been thought to be pancreatic specific. Flanagan suggested that part of the material previously thought to be of pancreatic origin may be derived from the gut, and that until the characteristics of tissue and circulating GLI are further defined the glucagon levels in circulation should be referred
to as either N-terminal or C-terminal reactive GLI. This convention is used in the present study.

**Iodination of glucagon**

The following additions to the iodination vessel were made with Hamilton syringes.

<table>
<thead>
<tr>
<th>Addition</th>
<th>Volume</th>
<th>Volume Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 mCi $^{125}$I</td>
<td>10 µl</td>
<td>10 µl (1 mg in 1.25 ml 0.01 N NaOH)</td>
</tr>
<tr>
<td>20 µg glucagon</td>
<td>25 µl</td>
<td>25 µl</td>
</tr>
<tr>
<td>0.4 M PO₄ buffer pH 7.2</td>
<td>25 µl</td>
<td></td>
</tr>
<tr>
<td>20 µg chloramine T</td>
<td>5 µl</td>
<td>5 µl (40 mg to 10 ml 0.2 M PO₄ buffer)</td>
</tr>
<tr>
<td>Mixed for 20 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Na₂S₂O₅</td>
<td>20 µl</td>
<td>20 µl (24 mg in 10 ml 0.2 M PO₄ buffer)</td>
</tr>
<tr>
<td>Mixed for 45 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KI</td>
<td>50 µl</td>
<td>50 µl (100 mg in 10 ml 0.05 M PO₄ buffer pH 7.4)</td>
</tr>
<tr>
<td>Mixed 20 seconds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2M Tris</td>
<td>5 µl</td>
<td></td>
</tr>
</tbody>
</table>

The iodination mixture was separated on a 25 x 1 cm column of QAE Sephadex A 25. The column was prepared by putting 2 ml of 1% Lister albumin made up in buffer. The column was set up in the cold room at 4°C and after application of the iodination mix buffer was pumped through at the rate of 9 ml per hour. One hundred 1 ml fractions were collected. 100 µl aliquots of each of the fractions were counted for radioactivity. The peak of activity occurred at fraction 60. Fractions 54–66 were pooled, diluted with 3 volumes of acid alcohol and stored in 0.5 ml fractions at -20°C. The labelled glucagon was further diluted 1 in 200 in 0.04 M phosphate buffer prior to assay.
Assay procedure

1 ml of 0.04M phosphate buffer was added to each plasma extract and swirled gently.

All procedures were carried out in trays of crushed ice. To each IP3 tube was added the following:

- 50 μl Trasylol
- 100 μl sample or standard or buffer (zero tubes)
- 100 μl buffer
- 100 μl antibody or buffer for non-specific tubes

The tubes were then incubated for 12 hours at 4°C

100 μl labelled glucagon was added to each tube

The tubes were then incubated at 4°C for 48 hours.

Each was set up in duplicate.

In each assay the following were always included:

- 2 x 100% $^{125}$I glucagon tubes: i.e. 100 μl diluted $^{125}$I glucagon for reference total counts.
- 4 buffer non-specific tubes: i.e. tubes in which 100 μl of phosphate buffer is substituted for antibody and 100 μl of buffer if substituted for the sample or standard. This allows a check on the % of non-specific binding to charcoal during separation.
- 4 zero tubes: i.e. tubes with 100 μl of buffer instead of sample or standard.
- 7 standards in duplicate: the standard curve.
- 2 pancreatic recovery tubes: i.e. 100 μl pancreatic recovery extract substituted for standard or sample.
- 2 gut recovery tubes: i.e. 100 μl gut recovery extract substituted for standard or sample.
- 2 sample non-specific tubes: i.e. tubes with 100 μl buffer instead of antibody

For each series of samples from each patient 2 non-specific tubes were set up with sample but no antibody.
2 sample tubes: for each sample.

The bound and free glucagon were separated using dextran-coated charcoal (10 g activated charcoal (Sigma) + 1 g dextran T-70 (Pharmacia) made up to 1 litre with 0.05 M phosphate buffer).

A 1% stock charcoal suspension was made as follows:

1 g Dextran T-70 (Pharmacia)
10 g activated charcoal (Sigma)
Made up to 1 litre with 0.05 M phosphate buffer.

Just prior to separation of each assay 1 litre of stock solution was diluted with 1 litre of 0.05 M phosphate buffer (0.5% charcoal solution).

1 ml of 0.5% charcoal was added to each tube. The tubes were left for 10 minutes on ice then centrifuged at 4°C for 20 minutes at 4000 r.p.m. The supernatants were poured off and discarded. The pellets were retained in the tubes and counted for radioactivity in a Wallac gamma counter.
INSULIN ASSAY

Plasma samples were assayed without prior extraction by a double antibody radioimmunoassay developed by Dr. D.S. Turner (University of Surrey).

Buffer

Oxoid barbitone acetate buffer for electrophoresis pH 8.6 was made up as follows:

- 8.25 g barbitone buffer
- + 625 mg dried human albumin (Blood Products Laboratory, Lister Institute)
- Made up to 500 ml with distilled water

The buffer was kept at 4°C while the assay was being set up.

Standards

Wellcome Human Insulin Standard was dissolved in 2 ml of buffer and stored as 0.25 ml aliquots at -20°C. On the day of the assay a daily working standard containing 1000 μU/ml of insulin was made up from one aliquot with barbitone buffer. The standard curve was set up by adding varying volumes of the working standard, with an Agla micrometer syringe, to give tubes containing 200, 150, 125, 100, 75, 50, 30, 20, 10 μU insulin/ml. The working standard was discarded after use.

Antibodies

First antibody - Wellcome Anti-insulin serum (guinea pig) was used. To each vial was added 15 ml of buffer.

Second antibody - Wellcome Anti-guinea pig (rabbit) precipitating serum was diluted 1 in 5 with buffer.
Iodinated insulin

Wellcome $^{125}$I insulin was diluted with buffer to give approximately 30,000 counts/100 µl/5 minutes.

Complement

Better separation of the assay was achieved by adding more guinea pig serum (complement) before the second antibody because a heavier and more substantial precipitate was obtained.

Wellcome complement was dissolved in 7 ml of buffer and stored in 0.5 ml aliquots at -20°C. For each assay aliquots were diluted 1 in 25 with buffer.

Assay procedure

The assay was carried out at 4°C on a cool tray which maintains the temperature of the tubes at 4°C.

To each tube was added:

Buffer - varied quantities for standard curve, 300 µl for samples. 50 µl sample or varied quantities of standard, the buffer + standard volume = 350 µl.

100 µl $^{125}$I insulin.
The tubes were mixed on a Whirlimixer and left overnight at 4°C.

50 µl complement.

100 µl anti-guinea pig precipitating serum.
The tubes were mixed on a Whirlimixer and left overnight at 4°C.

Each assay tube was set up in duplicate.
Each assay included a standard curve, a quality control sample of known insulin content, and unknown samples.
The bound and free insulin were separated by centrifugation. After the second incubation the tubes were spun at 4°C and 2500 r.p.m. for 30 minutes. The supernatant was poured off and the pellet retained in the tube was counted for radioactivity on a Wallac gamma counter. Five minute counts were usually made.

The standard curve counts were plotted against concentration and unknown samples were read off the standard curve.
APPENDIX B

COMPUTER PROGRAMME RSK4

12.57.05 + LIST GLUC
0.0 TYPE " PROGRAM RSK4 (05/08/75)"
0.1 LINE
0.2 TYPE "COMPUTES AREAS UNDER SAMPLE CURVES FOR"
0.3 TYPE "GLUCOSE & INSULIN DATA AND THE INS/GLU"
0.4 TYPE "RATIO."
0.5 TYPE "NEGATIVE VALUES ARE TAKEN AS MISSING"
0.6 TYPE "AND ARE REPLACED BY LINEAR ESTIMATES."
0.7 TYPE "LIMITS OF INTEGRATION ARE SET IN PART 8"
0.8 TYPE "AS 60 MIN AND 120 MIN."
0.85 LINE
0.9 TYPE "DOING PART 1"
0.95 DO PART 1
1.0 DEMAND N AS "NO OF SAMPLES"
1.1 TO STEP 20.00 IF N<3
1.2 TYPE "ENTER THE SAMPLE TIMES"
1.3 DEMAND T(L) FOR L=1(1)N
1.4 TYPE "DOING PART 2"
1.5 DO PART 2
2.0 TYPE "ENTER THE SAMPLE VALUES"
2.2 DO PART 14 FOR L=1(1)N
2.3 DO PART 3 FOR K=1(1)3
2.4 DO PART 6
2.5 LINE
2.55 TYPE FORM 5
2.59 LINE
2.6 TYPE ' GLUCOSE'
2.65 DO PART 8 FOR K=1
2.69 LINE
2.7 TYPE ' INSULIN'
2.75 DO PART 8 FOR K=2
2.79 LINE
2.8 TYPE ' INS/GLU'
2.85 DO PART 8 FOR K=3
2.89 LINE,2 TIMES
2.9 STOP
3.0 TO STEP 20.1 IF V(K,1)<0
3.1 DO PART 4 FOR J=2(1)N-1
4.0 DONE IF V(K,J)>=0
4.1 L=J-1
4.2 DO PART 5
4.3 V(K,J)=(T(J)-T(L))/(T(U)-T(L))
4.4 V(K,J)=V(K,J)*(V(K,U)-ABS(V(K,L)))+ABS(V(K,L))
4.5 V(K,J)=-V(K,J)
5.0 U=J+1
5.1 DONE IF V(K,U)<>0
5.2 U=U+1
5.3 TO STEP 20.2 IF U>1
5.4 TO STEP 5.1
6.0  LINE
6.1  TYPE " INPUT DATA"
6.3  TYPE FORM 1
6.4  DO PART 7 FOR L=1(1)N
6.5  LINE
7.0  TYPE L,T(L),V(1,L),V(2,L),V(3,L) IN FORM 2
8.0  DO PART 10 FOR U=60
8.1  DO PART 9
8.2  B=A
8.3  DO PART 10 FOR U=120
8.4  DO PART 9
8.5  TYPE A/B,A/(2*B) IN FORM 4
9.0  TYPE U,A,A/U IN FORM 3
10.00 SET A=0
10.05 SET L=1
10.1  TO STEP 10.9 IF U<T(L)
10.15  J=L+1
10.20  TO STEP 10.50 IF U>T(J)
10.25  TO STEP 10.60 IF U=T(J)
10.30  DO PART 12
10.35  TO STEP 10.65
10.50  DO PART 11
10.55  TO STEP 10.15
10.60  DO PART 11
10.65  DONE
10.90  TYPE "ERROR IN TIME LIMITS"
11.0  X=ABS(V(K,J))
11.1  Y=T(J)
11.2  DO PART 13
11.3  L=J
12.0  Y=U
12.1  X=(ABS(V(K,J))-ABS(V(K,L)))/(T(J)-T(L))
12.2  X=X*(Y-T(L))+ABS(V(K,L))
12.3  DO PART 13
13.0  A=A+(ABS(V(K,L))+X)/2*(Y-T(L))
14.0  TYPE L,T(L) IN FORM 6
14.1  DEMAND V(1,L) AS 'GLU'
14.2  DEMAND V(2,L) AS 'INS'
14.3  V(3,L)=V(2,L)/V(1,L)
14.4  SET V(3,L)=-1 IF (V(2,L)<0 OR V(1,L)<0)
20.0  TYPE "ERROR: TOO FEW SAMPLES"
20.05  TO STEP 20.9
20.1  TYPE "ERROR: NO INITIAL SAMPLE VALUE"
20.15  TO STEP 20.9
20.2  TYPE "ERROR: UNABLE TO INTERPOLATE"
20.9  STOP
FORM 1:
NO TIME GLUCOSE INSULIN INS/GLU
FORM 2:
###  ...........  ...........  ...........
FORM 3:
###  ...........  ...........
FORM 4:
RATIO  ...........  ...........
FORM 5:
TIME AREA WTD MEAN
FORM 6:
SAMPLE  #  # #
LSO:
DO PART C
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References (continued/20)


References (continued/21)


