Chapter 4

Metabolic consequences of overweight, underweight and physical activity/inactivity

Humans

Both body weight and physical activity can have marked effects on several physiological systems that may subsequently affect cancer risk. These include in particular effects on hormonal milieu and immune function. In this section, results from cross-sectional and human intervention studies on the relationships of weight, weight loss and physical activity with hormonal and immunological variables are reviewed. Both acute effects of physical activity sessions and long-term effects of training are covered.

Results on the association of selected hormones and binding globulins with obesity and weight reduction, and with physical activity are summarized in Tables 19 and 20, respectively.

Insulin resistance

An essential action of insulin in the body is control of the uptake, synthesis and use of glucose. The action of insulin is reflected in the glycaemic curve, which is a plot of plasma glucose level against time after an oral glucose dose (the oral glucose tolerance test, OGTT, as used routinely for diabetes diagnosis). The area under the glycaemic curve provides a measure of the rate of disposal of glucose; insulin levels can be similarly plotted during the test and the area under the insulinaemic curve is also an indication of insulin sensitivity. Obesity and lack of physical activity are major determinants of insulin resistance.

Insulin resistance is a state of reduced responsiveness of muscle, liver and adipose tissue to insulin. One major effect of insulin resistance is a rise in blood glucose levels, due to lower glucose uptake from blood by skeletal muscle and liver, as well as to reduced insulin-related inhibition of hepatic gluconeogenesis (DeFronzo, 1988; Mandarino, 1999; Radziuk & Pye, 1999). This chronic increase in blood glucose concentration is also reflected in higher levels of glycosylated haemoglobin (Larsen, 1989). At the extreme, insulin resistance may cause glucose intolerance and lead to the development of (type II) diabetes mellitus (Unger & Foster, 1998). In insulin-resistant states, fasting and non-fasting plasma insulin levels increase so as to maintain blood glucose levels within acceptable limits.

Elevated fasting and postprandial insulin concentrations, increased glycosylated haemoglobin and decreased glucose tolerance are indisputably associated with obesity (Haffner *et al.*, 1994a; Strain *et al.*, 1994; Ivandic *et al.*, 1998; Boeing *et al.*, 2000). The relationship between BMI and fasting insulin level is continuous and linear: for example, in the study of Strain *et al.* (1994), fasting insulin concentration increased by 1 U/mL per unit increase in BMI. There is also good evidence that an increase in intra-abdominal (visceral) fat is especially related to the development of insulin resistance (Krotkiewski *et al.*, 1983; Seidell *et al.*, 1990a; Leenen *et al.*, 1994; Rasmussen *et al.*, 1994; Cigolini *et al.*, 1995; Björntorp, 1997; Anderssen *et al.*, 1998; Ivandic *et al.*, 1998; Haffner, 2000).

One central mechanism linking particularly visceral fat accumulation to insulin sensitivity is an increase in plasma concentrations of free fatty acids (Figure 26). (Seidell *et al.*, 1990a; Haffner, 2000). Intra-abdominal fat is mobilized more easily than most fat deposits elsewhere in the body, because it is more sensitive to lipolytic stimuli (e.g., through β3-adrenergic receptors), leading to a constantly increased release of free (non-esterified) fatty acids into the circulation. Elevation of free fatty acid levels causes liver and skeletal muscle to shift towards greater oxidation of fatty acids for energy production, and a relative inhibition of enzymes in the glycolytic cascade (Krebs cycle) (Randle, 1998). As a consequence, the capacity of these tissues to absorb and metabolize glucose and to store glucose in the form of glycogen decreases (Ebeling & Koivisto, 1994) and cells accumulate more triglycerides instead of glycogen as a local energy reserve. In addition to the altered activities of glucose and fatty acid-metabolizing enzymes, insulin-resistant cells generally have lower membrane levels of insulin receptors (Flier, 1983).

Insulin resistance and fasting plasma insulin concentrations reach peak levels during the pubertal growth spurt (Amiel *et al.*, 1986; Bloch *et al.*, 1987; Smith *et al.*, 1988; Caprio *et al.*, 1989). This may be due to the rise in pituitary secretion of growth hormone, which has strong lipolytic effects and increases plasma concentrations of free fatty acids (Bratusch-Marrain *et al.*, 1982; Rizza *et al.*, 1982; Hindmarsh *et al.*, 1988; Martha & Reiter, 1991). During puberty and adolescence, as at adult age, insulin resistance is

Table 19. Associations of obesity and weight reduction with selected hormones and binding globulins

Hormone or binding globulin	Obesity (cross-sectional)	Weight reduction
Insulin	↑	↓
Insulin-like growth factor-I (IGF-I)	↓ or NE; ↑ (free IGF-I)	↑ or NE
IGF binding protein-1	↓	↑ ?
IGF binding protein-3	↑ or NE	↑ ?
Human growth hormone	↓	↑
Sex-hormone binding globulin	↓	↑
Total testosterone	↑ (PCOS); ? (F); ↓ (M)	↓ or NE (F); ? (M)
Free testosterone	↑ (F) ; ↓ (M)	↓ or ↑ or NE
Estradiol	↑ (M, postmenopausal F)	↓
	NE ? (premenopausal F)	↓ or NE
Dehydroepiandrosterone (sulfate)	↓ or NE	↑ or NE
Prolactin	NE	NE

↑, increased levels; ↓, decreased levels; NE, no observed effect; ?, very uncertain; M, males; F, females.

Table 20. Associations of physical activity with selected hormones and binding globulins

Hormone or binding globulin	Acute effects	Chronic effects
Insulin	↓	↓
Insulin-like growth factor-I (IGF-I)	↑ or NE	↑ or NE
IGF binding protein-1	↑	?
IGF binding protein-3	↑ or NE	↑ or NE
Human growth hormone	↑	↑ ?
Sex-hormone binding globulin	↑	?
Testosterone	↑ (also for free testosterone)	↓ or NE
Estradiol	↑ or ↓	↓ ? (F); NE (M)
Dehydroepiandrosterone (sulfate)	?	NE
Prolactin	↑	NE

Acute effects: assessment made during or immediately after physical activity; chronic effects: assessment made cross-sectionally (physically active vs inactive subjects) or longitudinally (effects of increased activity levels).
↑, increased levels; ↓, decreased levels; NE, no observed effect; ?, very uncertain; M, males; F, females.

generally aggravated by obesity (Travers *et al.*, 1995; Attia *et al.*, 1998).

Weight reduction, and particularly loss of visceral fat mass, leads to improved insulin sensitivity and to decreased serum insulin concentration, especially in subjects with impaired glucose tolerance (Leenen *et al.*, 1994; Strain *et al.*, 1994; Colman *et al.*, 1995; Rasmussen *et al.*, 1995; Dengel *et al.*, 1996; Torjesen *et al.*, 1997; Turcato *et al.*, 1997; Fogelholm *et al.*, 2000b; Ross *et al.*, 2000a). Weight regain, often observed after weight reduction, is followed by an impairment in insulin action (Fogelholm *et al.*, 2000b). A few studies have shown a decrease in insulin levels along with weight loss during or shortly after the intervention period, but a rebound of insulin concentrations afterwards, even though weight was maintained at a reduced level (Fogelholm *et al.*, 2000b).

Independently of the effect of excess body fat, lack of physical activity level may also contribute to the development of insulin resistance. Relationships between physical activity and indices of insulin sensitivity have been extensively investigated and reviewed (e.g. Schwartz, 1990; Bonen, 1995; Ivy, 1997;

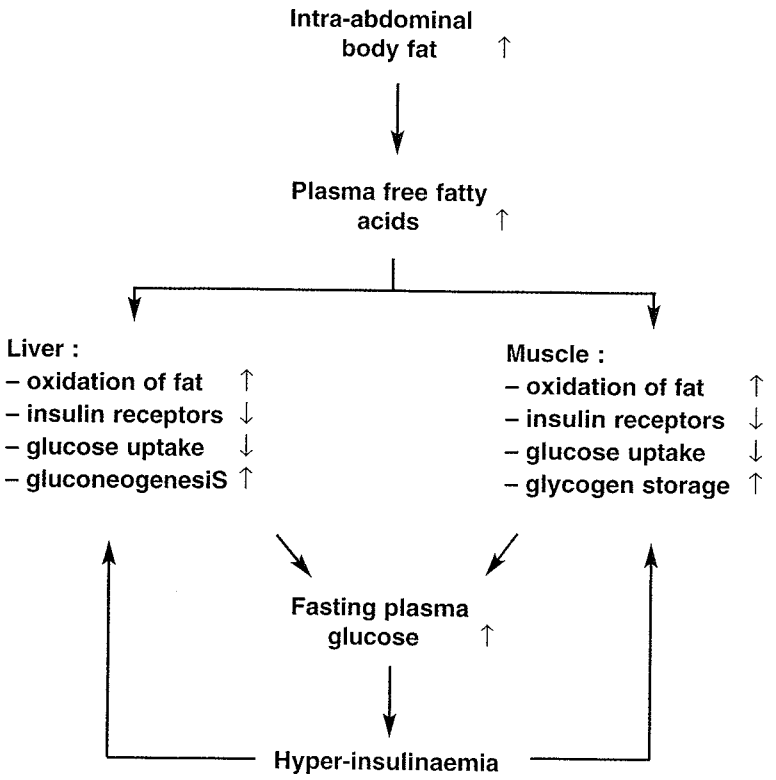

Figure 26 Effects of obesity on insulin sensitivity
Modified from Kaaks (1996)

Godsland *et al.*, 1998; Thune *et al.*, 1998; Grimm, 1999; Kelley & Goodpaster, 1999; van Baak & Borghouts, 2000; Raastad *et al.*, 2000). Cross-sectional studies have shown that regular physical activity and aerobic fitness are associated with better insulin sensitivity. In non-insulin-dependent diabetes mellitus (NIDDM) patients, physical fitness is inversely related to modest but favourable changes in glycosylated haemoglobin and glucose tolerance (Albright *et al.*, 2000).

To some extent, the effects of regular physical activity on insulin sensitivity may be mediated by reductions in body fat deposits. Regular aerobic exercise has been shown in a few studies to cause a preferential reduction in intra-abdominal fat in both men and women (Després *et al.*, 1991; Schwartz *et al.*, 1991; Buemann & Tremblay, 1996), but these data are not conclusive. However, apart from any such effect, physical activity has been shown to decrease insulin resistance and plasma insulin levels by more rapid mechanisms that may be relatively independent of changes in body weight and composition (Borghouts & Keizer, 2000; van Baak & Borghouts, 2000). Cross-sectional studies have shown inverse associations between physical activity levels and indices of insulin sensitivity independent of body size (Lindgarde & Saltin, 1981; Wang *et al.*, 1989; Regensteiner *et al.*, 1991; Feskens *et al.*, 1994; Borghouts & Keizer, 2000). Possible mechanisms unrelated to changes in overall adiposity include reduction of intramuscular stores of triglycerides (Pan *et al.*, 1997a), increased muscular phosphatidylinositol-3 kinase activity (Houmard *et al.*, 1999) and an increased capacity of skeletal muscle to metabolize or store glucose (Perseghin *et al.*, 1996; Hargreaves, 1998; Goodyear & Kahn, 1998). In addition, there are increases in insulin receptor density (Ivy, 1997) and in the number of glucose transporters (e.g., GLUT-4) in muscle cell membranes, and improved intrinsic activity of the glucose transporters (Bonen, 1995; Perseghin *et al.*, 1996; Grimm, 1999; van Baak & Borghouts, 2000).

Human intervention studies show both acute and long-term effects of physical activity on insulin sensitivity and plasma insulin concentrations. Because impaired glucose tolerance is more prevalent in the aged, it is not surprising that many interventions on the effects of exercise training and insulin action have been conducted with elderly subjects (Grimm, 1999; Kelley & Goodpaster, 1999). Even a single session of sustained submaximal physical activity improves insulin sensitivity and reduces plasma insulin levels for up to 48 hours (Perseghin *et al.*, 1996; Kraemer *et al.*, 1999; van Baak & Borghouts, 2000). However, although insulin action is improved acutely following an exercise session, long-term improvements in glucose tolerance due to moderate physical activity accrue only slowly (Bonen, 1995).

Randomized controlled trials have shown that a programme of aerobic exercise (three times weekly), with a duration of 6–10 months and without a concomitant decrease in body weight, improves insulin action, as shown by decreased area under the glycaemic curve during an oral glucose tolerance test. Many, but not all (Poehlman *et al.*, 1994), studies also show decreased

fasting serum insulin concentrations. Most non-randomized trials with obese, insulin-resistant subjects used aerobic exercise as the training mode (Kelley & Goodpaster, 1999), with results similar to those from randomized trials. Eriksson *et al.* (1997) showed that resistance training also may improve glucose tolerance.

Because of the wide diversity of types of exercise, training volume and subject characteristics, it is not possible to draw firm conclusions on, for instance, a possible dose-dependence of the response. The effect of exercise intensity is also uncertain, but the present view is that even exercise of moderate intensity is beneficial in terms of insulin sensitivity (van Baak & Borghouts, 2000).

It should be noted that the effects of exercise *per se* on insulin action, without a concomitant weight change, are more modest than the changes seen after weight reduction (Torjesen *et al.*, 1997; Ross *et al.*, 2000a). However, the effects of exercise and diet together on insulin action may be more effective than the effects of diet alone (Dengel *et al.*, 1996), even though the added exercise programme usually does not increase the loss of body weight. In a study by Ross *et al.* (2000a), the improvement in glucose disposal after a 7.5-kg weight reduction was slightly, but not significantly, better when weight reduction was achieved solely by exercise, compared with a traditional low-energy diet. These results indicate that weight loss and exercise affect insulin metabolism through different mechanisms (van Baak & Borghouts, 2000).

IGF-I and IGF-binding proteins

Insulin and insulin-like growth factor (IGF)-I are central to the regulation of anabolic (growth) processes as a function of available energy and essential nutrients (e.g., amino acids) from body reserves and diet (Straus, 1994; Thissen *et al.*, 1994; Estivariz & Ziegler, 1997; Yu & Berkel, 1999; Kaaks & Lukanova, 2001). Moreover, insulin and IGF-I also act as key regulators of the synthesis and biological availability of sex steroids by stimulating steroidogenesis (Kaaks, 1996; Poretsky *et al.*, 1999; Kaaks *et al.*, 2000a), while inhibiting the hepatic synthesis of sex-hormone-binding globulin (SHBG) (Plymate *et al.*, 1988; Singh *et al.*, 1990; Pugeat *et al.*, 1991; Crave *et al.*, 1995a). Alterations in levels of insulin or in IGF-I bioactivity thus provide an important physiological link between energy balance, physical activity and levels of bioavailable androgens and estrogens (Pugeat *et al.*, 1991; Erfurth *et al.*, 1996; Pfeilschifter *et al.*, 1996).

IGF-I and at least six different IGF-binding proteins (IGFBPs) are synthesized in most, perhaps all, organ systems; however, most (> 80%) of the IGF-I and IGFBPs in the circulation is synthesized in the liver. IGF-I bioactivity is the overall result of the endocrine, paracrine and autocrine effects of IGF-I and IGFBPs on cellular receptors. It is generally believed that IGF-I bioactivity increases when absolute plasma and tissue concentrations of IGF-I rise. However, IGF-I bioactivity is strongly modulated by IGFBPs, which control the efflux of IGF-I from the circulation towards target tissues and, within tissues, regulate binding of IGF-I to its tissue receptors (Jones & Clemmons, 1995; Wetterau *et al.*, 1999).

In the circulation, more than 90% of IGF-I is bound to IGFBP-3, plus another glycoprotein called acid-labile subunit (ALS). Another small fraction is bound to IGFBP-5, which also forms a ternary complex with ALS, or to the IGFBPs -1, -2, -4 and -6. Because of the very high affinities of IGFBP-3 and IGFBP-5 for IGF-I, and their large complexes with ALS, IGF-I bound to IGFBP-3 or IGFBP-5 cannot diffuse through the endothelial barrier. The IGFBPs -1, -2, -4 and -6 are smaller (hence can diffuse from the circulation into the extravascular space), and have lower affinity for IGF-I. Therefore, it is believed that a reduction in IGFBP-3, with a transfer of IGF-I towards the latter IGFBPs, increases IGF-I bioavailability. Furthermore, reductions in circulating IGFBP-1 or IGFBP-2 have been found to be associated with an increase in the small fraction of plasma IGF-I unbound to any binding protein (referred to as 'free IGF-I'), and hence are also believed to increase IGF-I availability.

At the tissue level, the IGFBPs have been proposed mostly to inhibit binding of IGF-I to its receptor. Nevertheless, studies *in vitro* have shown that some IGFBPs may also enhance IGF-I binding to its receptors, depending on the relative concentrations of IGF-I and IGFBPs. These modulating effects of the IGFBPs may be altered by phosphorylation of IGFBPs or by enzymatic proteolysis (Jones & Clemmons, 1995). IGFBP-3 has been shown to exert pro-apoptotic and antimitogenic effects through a specific IGFBP-3 binding site on the membranes of mammary, prostatic, endometrial or colonic cells (Ferry *et al.*, 1999; Baxter, 2000).

Circulating levels of growth hormone (GH), IGF-I, and IGFBP-3 reach peak levels during the pubertal growth spurt, but then gradually decrease with age. Conversely, levels of IGFBP-1 and IGFBP-2 are lower during puberty than at later ages.

Levels of IGF-I and of IGFBPs -1, -2 and -3 vary strongly with changes in energy intake and body energy (fat) stores. Nutritional regulation of levels of IGF-I and IGFBPs -1, -2 and -3 is effectuated largely along two relatively independent physiological axes – one for GH and one for insulin (Straus, 1994; Thissen *et al.*, 1994; Kaaks & Lukanova, 2001). GH provides the principal stimulus for synthesis of IGF-I and of IGFBP-3 (Figure 27). This stimulatory effect by GH, however, is modulated by insulin, which stimulates the synthesis of GH receptors (Baxter & Turtle, 1978; Leung *et al.*, 1997, 2000) and favours cellular amino acid uptake for protein synthesis.

Furthermore, insulin directly inhibits the synthesis of IGFBP-1 (Suikkari *et al.*, 1988; Conover & Lee, 1990; Jones & Clemmons, 1995) and is also inversely related to circulating IGFBP-2 levels (Wabitsch *et al.*, 1996; Argente *et al.*, 1997a; Nam *et al.*, 1997).

Through these mechanisms, conditions of low pancreatic insulin production, such as chronic fasting (Clemmons *et al.*, 1981; Caufriez *et al.*, 1984), energy-protein malnutrition (Clemmons & Underwood, 1991; Thissen *et al.*, 1994), anorexia nervosa (Tanaka *et al.*, 1985; Counts *et al.*, 1992; Hochberg *et al.*, 1992; Golden *et al.*, 1994), but also insulin-dependent diabetes mellitus (IDDM) (Bereket *et al.*, 1995, 1999) cause resistance of liver and other tissues to the GH stimulus, and hence a dramatic drop in absolute levels of IGF-I and IGFBP-3. In addition, levels of IGFBP-1 and IGFBP-2 rise. As an overall result, the bioavailability of IGF-I to tissue receptors decreases, and this is also reflected by a reduction of plasma free IGF-I (Thissen *et al.*, 1994; Kaaks & Lukanova, 2001).

In contrast, under conditions of elevated endogenous insulin production, such as obesity, NIDDM and other insulin-resistant states, tissues are optimally responsive to the GH stimulus, so that smaller amounts of GH are needed to stimulate IGF-I synthesis. Furthermore, conditions related to chronic hyperinsulinaemia reduce levels of IGFBP-1 (Clemmons & Underwood, 1991; Thissen *et al.*, 1994; Frystyk *et al.*, 1995; Wabitsch *et al.*, 1996; Argente *et al.*, 1997b; Nam *et al.*, 1997) and IGFBP-2 (Wabitsch *et al.*, 1996; Argente *et al.*, 1997b; Nam *et al.*, 1997). As a consequence, plasma free IGF-I levels increase (Frystyk *et al.*, 1995; Nam *et al.*, 1997; Nyomba *et al.*, 1997; Attia *et al.*, 1998).

Paradoxically, however, obesity does not increase absolute plasma IGF-I levels, but generally does not change or leads to a mild reduction in IGF-I concentrations compared with those in the normally nourished but non-insulin-resistant state (Copeland *et al.*, 1990; Conover *et al.*, 1992; Rasmussen *et al.*, 1995; Morales *et al.*, 1996; Goodman-Gruen & Barrett-Connor, 1997; Attia *et al.*, 1998; Saitoh *et al.*, 1998). Three studies have shown that low IGF-I levels are more strongly related to visceral than to subcutaneous or total fat mass (Mårin *et al.*, 1993; Rasmussen *et al.*, 1994; De Pergola *et al.*, 1998). However, more data are needed to confirm the relationships between fat distribution and IGF-I.

The mild decrease in absolute IGF-I concentrations in obese and hyperinsulinaemic subjects can be explained by an increased negative feedback of plasma free IGF-I on pituitary GH secretion (Tannenbaum *et al.*, 1983; Chapman *et al.*, 1998). Basal GH levels, as well as

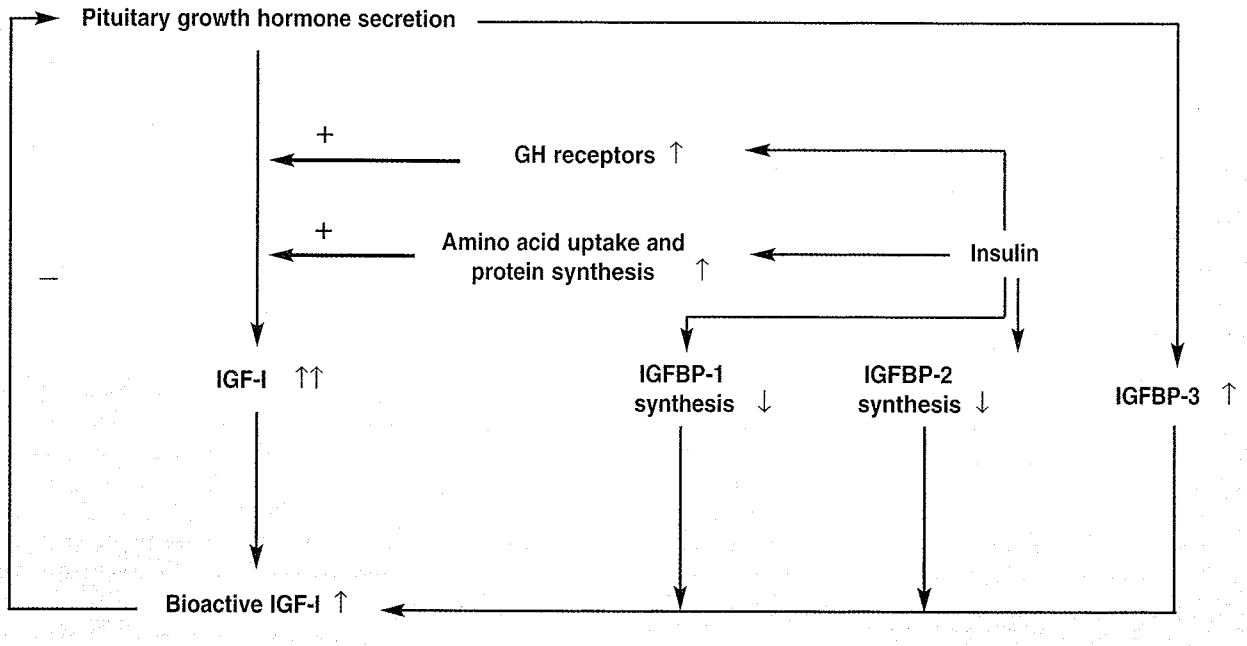

Figure 27 Regulation of the bioactivity of insulin-like growth factor (IGF)-I by growth hormone (GH) and insulin.
+, stimulating effect; –, inhibitory effect; ↑, increased; ↓, decreased
Kaaks *et al.* (2000b)

GH secretion stimulated by various physiological stimuli, have been shown to be lower in obese subjects (Iranmanesh *et al.*, 1991; Veldhuis *et al.*, 1995; Rasmussen *et al.*, 1995; Morales *et al.*, 1996; Bona *et al.*, 1999; Micic *et al.*, 1999). This decrease appears to be due to reductions both in growth hormone burst frequency (Veldhuis *et al.*, 1991; Morales *et al.*, 1996) and in burst mass (Veldhuis *et al.*, 1995). In addition, GH levels may also be decreased by an increased rate of clearance (Iranmanesh *et al.*, 1991; Veldhuis *et al.*, 1991; Langendonk *et al.*, 1999).

Data on the relationship between IGFBP-3 and obesity are conflicting, so that no firm conclusions can be drawn. Overall, there appears to be no strong association of obesity with IGFBP-3 levels (Rasmussen *et al.*, 1994; Morales *et al.*, 1996; Falorni *et al.*, 1997; Attia *et al.*, 1998; Saitoh *et al.*, 1998).

Weight reduction leads to increased serum GH levels and to restored responses to physiological stimuli (Gama *et al.*, 1990; Tanaka *et al.*, 1990; Rasmussen *et al.*, 1995). Moreover, growth hormone-binding protein (GHBP) levels decrease after weight loss. In one study (Rasmussen *et al.*, 1996), changes in waist circumference and abdominal sagittal diameter during weight loss were the major determinants of the fall in GHBP. Short-term hypo-energy feeding did not affect GHBP (Rasmussen *et al.*, 1996), suggesting that the changes observed were due to changes in fat mass rather than, for example, insulin availability.

Data on the effects of weight reduction on IGF-I are conflicting. Some studies have showed an expected increase in total IGF-I during weight reduction (Poulos *et al.*, 1994; Rasmussen *et al.*, 1994, 1995), but others saw no response (Gama *et al.*, 1990; Falorni *et al.*, 1997) or even a decrease (Smith *et al.*, 1995; De Pergola *et al.*, 1998). The conflicting findings may be caused partly by varying IGF-I responses to large variations in energy and protein intake (Smith *et al.*, 1995; De Pergola *et al.*, 1998; Ross, 2000). In addition, results may vary according to whether one measures an acute effect during the weight reduction phase or afterwards, and may depend on the subject's degree of obesity at start. Weight reduction has been shown to increase plasma concentrations of IGFBP-1 (Kiddy *et al.*, 1989; Musey *et al.*, 1993; Hellénius *et al.*, 1995) and IGFBP-2 (Clemmons *et al.*, 1991; Thissen *et al.*, 1994; Wabitsch *et al.*, 1996).

Cross-sectional studies comparing baseline (resting) levels of IGF-I between trained and untrained individuals are few, and do not allow any firm conclusions. They have covered both aged and young adult subjects, but the results do not indicate any age-by-physical-activity interactions. After proper adjustment for age and body composition, these studies did not show any difference between IGF-I levels of trained and untrained participants (Goodman-Gruen & Barrett-Connor, 1997; Walker *et al.*, 1999). However, Poehlman and Copeland (1990) reported higher IGF-I concentrations in physically active than in inactive subjects.

Numerous small human intervention studies (Bang *et al.*, 1990; Cappon *et al.*, 1994; Koistinen *et al.*, 1996; Schwarz *et al.*, 1996; Hornum *et al.*, 1997; Nguyen *et al.*, 1998; Bermon *et al.*, 1999; Chadan *et al.*, 1999; Wallace *et al.*, 1999; Elias *et al.*, 2000), with some exceptions (Kraemer *et al.*, 1992, 1995; Hopkins *et al.*, 1994; Schmidt *et al.*, 1995; Di Luigi *et al.*, 1997; Bonnefoy *et al.*, 1999), have shown an acute but transient increase in levels of IGF-I immediately during and after a bout of exercise. The IGF-I response may be related to the intensity and duration of exercise: Nguyen *et al.* (1998) reported increased IGF-I after short-term (20 min) ergometer exercise, a decrease after prolonged (3 h) endurance exercise and no change after interval training. The increase in IGF-I may be explained by an acute rise in pituitary GH secretion (Cappon *et al.*, 1994; Kraemer *et al.*, 1995; Schmidt *et al.*, 1995; Di Luigi *et al.*, 1997; Hornum *et al.*, 1997; Wideman *et al.*, 1999). Nevertheless, one study in peripubertal children and adolescents, who have the highest plasma IGF-I levels compared to other age groups, showed a significant reduction in IGF-I after a short bout of exercise (Scheett *et al.*, 1999) or after an increase in physical activity levels for three days. The serum concentration of the main binding protein of IGF-I, namely IGFBP-3, seems to increase during exercise, if a simultaneous rise in IGF-I is observed (Schwarz *et al.*, 1996; Nguyen *et al.*, 1998).

More prolonged episodes of intense exercise for several hours, such as running a marathon, have also been found to decrease circulating IGF-I as well as IGFBP-3 levels (Koistinen *et al.*, 1996). This suggests that decreases in absolute IGF-I levels may be achieved only by more prolonged exercise, and possibly this is so only when exercise leads to a (transient) negative energy balance.

A very consistent acute effect of more prolonged bouts of physical exercise is a large (up to tenfold or even higher) increase in IGFBP-1 (Hopkins *et al.*, 1994; Koistinen *et al.*, 1996; Schwarz *et al.*, 1996; Nguyen *et al.*, 1998; Wallace *et al.*, 1999; Scheett *et al.*, 1999). The IGFBP-1 response may be smaller, however, if exercise intensity is not high enough (Schwarz *et al.*, 1996). The increase in IGFBP-1 appears to be independent of variations in insulin but, instead, is most likely due to an exercise-induced rise in cortisol levels. Cortisol increases the synthesis and plasma levels of IGFBP-1 (Unterman, 1993; Lee *et al.*, 1997b; Katz *et al.*, 1998), an effect opposite to that of insulin.

Longer-term increases in physical exercise (regular training) over periods of weeks to several months have also led to increases in IGF-I levels in elderly

subjects (Poehlman *et al.*, 1994; Bonnefoy *et al.*, 1999), as well as in younger men and women (Roelen *et al.*, 1997; Koziris *et al.*, 1999), although several other studies in elderly men showed no clear effect on IGF-I (Nicklas *et al.*, 1995; Vitiello *et al.*, 1997). In a weight-reduction study, inclusion of exercise prevented a diet-induced decrease in IGF-I concentration (Hellénius *et al.*, 1995). The effects of resistance training on IGF-I seem to be negligible (Kraemer *et al.*, 1999).

Data on the effects of regular training on IGFBP-3 levels are limited: both increased (Koziris *et al.*, 1999) and maintained (Poehlman *et al.*, 1994) IGFBP-3 concentrations have been reported.

Taken together, although physical activity leads to an acute increase in absolute IGF-I levels, its effect on long-term average IGF-I concentrations is less clear. Furthermore, the effects of physical activity on IGFBP-3 concentrations and on IGF-I levels relative to IGFBP-3 are unclear.

Total and bioavailable sex steroids

Sex steroid hormones are essential for the growth, differentiation and function of many tissues in both men and women. This section covers the main human androgens (Δ4-androstenedione, testosterone, dehydroepiandrosterone (DHEA) and its sulfate (DHEAS)), estrogens (estrone, estradiol) and SHBG. In women, androgens are produced by the ovary (testosterone, androstenedione) as well as by adrenal glands (DHEA, DHEAS, androstenedione) (Stanczyk, 1997); in men the androgens are also produced both by the testes and adrenal glands. Estrogens (estrone, estradiol) are produced by the ovary in premenopausal women. After menopause, ovarian production of estrogens and of progesterone falls to very low levels, whereas that of androgens decreases more gradually with age. In postmenopausal women, the principal estrogen is estrone, produced by peripheral aromatization of androstenedione, mainly within adipose tissue. Adipose tissue is also an important source of estrogens in men.

Both overall and central adiposity have been associated with differences in total and bioavailable plasma sex steroid levels, in pre- and postmenopausal women as well as men. These relationships are mediated by a number of mechanisms (Figure 28). First, obesity leads to a state of relative insulin resistance, chronic hyperinsulinaemia and an increase in IGF-I bioactivity, due to insulin-mediated decreases in IGFBP-1 and IGFBP-2. Insulin and increased bioactive IGF-I, in turn, inhibit the hepatic synthesis of SHBG (Plymate *et al.*, 1988; Pasquali *et al.*, 1990; Singh *et al.*, 1990; Pugeat *et al.*, 1991; Crave *et al.*, 1995a; Nestler, 2000), a globulin that specifically binds sex hormones in the circulation. It is generally agreed that the unbound fraction determines the actual biological activity of androgens and estrogens (Enriori *et al.*, 1986). Second, insulin and IGF-I enhance the synthesis of sex steroids (androgens and estrogens) by the gonads and adrenal glands (Kaaks, 1996; Poretsky *et al.*, 1999; Kaaks *et al.*, 2000a). Third, in the adipose tissue compartment, androgens (Δ4-androstenedione, testosterone) are converted into estrogens (estrone, estradiol) by the enzyme aromatase (Siiteri, 1987; Azziz, 1989). Fourth, an increase in bioavailable androgens, unbound to SHBG, may lead to increased estrogen synthesis in adipose tissue. Fifth, for a given level of SHBG, an increase in plasma testosterone concentration tends to raise the level of bioavailable estradiol, because SHBG has a greater affinity for testosterone than for estradiol. One general consequence of these mechanisms, in both women and men, and irrespective of menopausal status, is a decrease in plasma SHBG level, which generally correlates inversely with plasma fasting insulin and IGF-I. However, the final consequences of these actions for levels of total and bioavailable sex steroids are

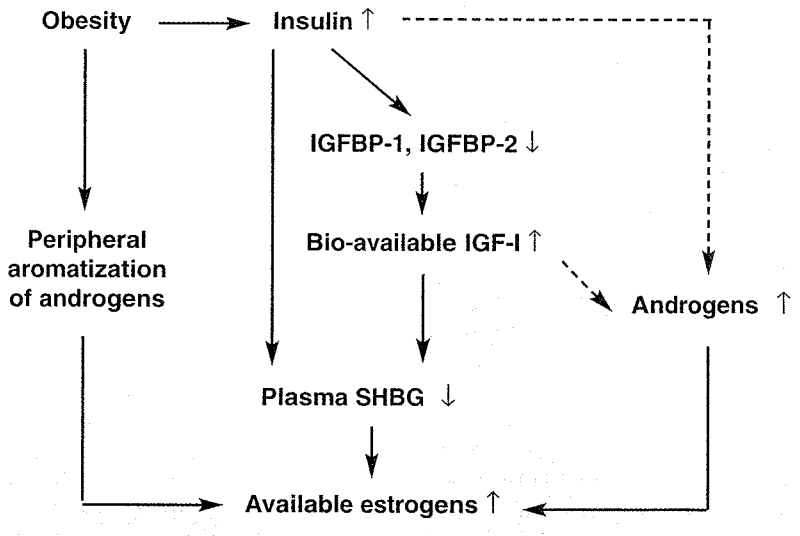

Figure 28 Mechanisms underlying relationships between overall and central adiposity and differences in total and bioavailable plasma sex steriod levels
↑ Increased levels, ↓ decreased levels, ----------, observed only in PCOS

not identical in men and women, and in women may also depend on the presence of genetic background factors that predispose to development of ovarian hyperandrogenism.

Sex hormone-binding globulin

The amount and distribution of body fat has very clear associations with blood SHBG concentration. In children and adolescents, obesity may be associated with low SHBG levels (Wabitsch *et al.*, 1995), although not all studies have found a significant relationship (de Ridder *et al.*, 1990). For girls, the inverse correlation is stronger with abdominal than gluteal obesity (Wabitsch *et al.*, 1995). Similar inverse correlations between BMI and SHBG are observed in both adult women (Kirschner *et al.*, 1990; Leenen *et al.*, 1994; Bernasconi *et al.*, 1996; Pasquali *et al.*, 1997a; Turcato *et al.*, 1997) and men (Field *et al.*, 1994; Haffner *et al.*, 1994a, 1997; Hautanen, 2000). Many (Kirschner *et al.*, 1990; Pasquali *et al.*, 1990; Kaye *et al.*, 1991; Leenen *et al.*, 1994; Ivandic *et al.*, 1998), but not all (Pasquali *et al.*, 1991; Haffner *et al.*, 1993a, 1994a; Mårin *et al.*, 1993; Hautanen, 2000), studies suggest that low SHBG levels are more tightly related to abdominal fatness than to general obesity. Use of different ways to measure fat distribution (e.g., waist-to-hip circumference ratio (WHR), computed tomography, magnetic resonance imaging, etc.) may have contributed to the inconsistencies.

The SHBG responses to weight reduction by any technique (diet, diet and exercise, drugs, surgical operation) are very straightforward: the concentration increases in adolescent girls (Wabitsch *et al.*, 1995) and in adult women (O'Dea *et al.*, 1979; Kopelman *et al.*, 1981; Kiddy *et al.*, 1989; Hamilton-Fairley *et al.*, 1993; Leenen *et al.*, 1994; Strain *et al.*, 1994; Crave *et al.*, 1995b; Turcato *et al.*, 1997) and men (Harlass *et al.*, 1984; Strain *et al.*, 1988, 1994; Guzik *et al.*, 1994; Leenen *et al.*, 1994; Vermeulen, 1996; Jakubowicz & Nestler, 1997; Tymchuk *et al.*, 1998). The reverse is also true: SHBG decreases in anorexia nervosa patients during refeeding and weight gain (Barbe *et al.*, 1993). Strain *et al.* (1994) estimated that SHBG levels increased by 0.43 nmol/L per unit decrease in BMI. They also noted that the slope of the change in SHBG versus change in BMI was steeper than the slope of the cross-sectional inverse relationship between BMI and SHBG. These findings may indicate that part of the increased SHBG is an acute response to the weight reduction technique (e.g., diet). An alternative explanation could be that during the dynamic phase of weight loss, SHBG is temporarily increased further than one would expect on the basis of the cross-sectional relationship, perhaps because of a stronger reduction in insulin levels during the intervention period than in the subsequent weight-maintenance phase.

The relationships between physical activity and SHBG are important in order to interpret the effects of physical activity on sex hormones. SHBG levels increase acutely, but only transiently, during physical exercise (Gray *et al.*, 1993; Zmuda *et al.*, 1996; Kraemer *et al.*, 1998). The long-term effects of physical activity are less clear, however. Cross-sectional studies have found either similar (Bagatell & Bremner, 1990) or higher (Tikkanen *et al.*, 1998) SHBG levels in trained than in untrained subjects. Human intervention studies have also shown conflicting results, with unchanged (Houmard *et al.*, 1994; Fahrner & Hackney, 1998; Häkkinen *et al.*, 2000), increased (Kumagai *et al.*, 1994) or decreased (Walker *et al.*, 1999) SHBG levels after prolonged training periods.

Androgens and estrogens

Regarding total and bioavailable plasma sex steroids, the effects of obesity and physical activity vary between men and women. In women, these effects depend also on menopausal status and on the presence or absence of functional ovarian hyperandrogenism or polycystic ovary syndrome (PCOS), a relatively frequent syndrome characterized by elevated plasma androgen levels, oligomenorrhoea and frequent anovulatory cycles, that affects 4–8% of premenopausal women (Figure 29).

Most studies of normo-androgenic premenopausal women have not shown any clear association between plasma insulin and absolute androgen concentrations (Evans *et al.*, 1983; Pasquali *et al.*, 1987; Amemiya *et al.*, 1990; Seidell *et al.*, 1990b; Weaver *et al.*, 1990; Austin *et al.*, 1991). Nevertheless, because of the decrease in plasma SHBG, obesity does increase levels of bioavailable androgens, unbound to SHBG, also in these women (de Ridder *et al.*, 1990; Kirschner *et al.*, 1990; Leenen *et al.*, 1994; Bernasconi *et al.*, 1996; Ivandic *et al.*, 1998; Penttilä *et al.*, 1999). In contrast, in normo-androgenic premenopausal women, obesity and chronic hyperinsulinaemia appear to have no or only little effect on levels of estradiol, measured either as absolute concentrations or as bioavailable levels unbound to SHBG. This might be explained by the relatively low estrogen production by adipose tissue, compared to the ovarian production. Another possible explanation is that in premenopausal women, total estrogen levels are maintained at relatively constant levels through feedback of estradiol on pituitary secretion of follicle-stimulating hormone (FSH). Kirschner *et al.* (1990) and Leenen *et al.* (1994) found that free estradiol levels in premenopausal women with abdominal (android) obesity were higher than in subjects with lower-body (gluteal) obesity. Moreover, abdominal obesity may be associated with higher estrone concentration (Pasquali *et al.*, 1990) in premenopausal women. Underweight, anorectic and eating-disordered premenopausal women have reduced concentrations of estradiol (Soygür *et al.*, 1996).

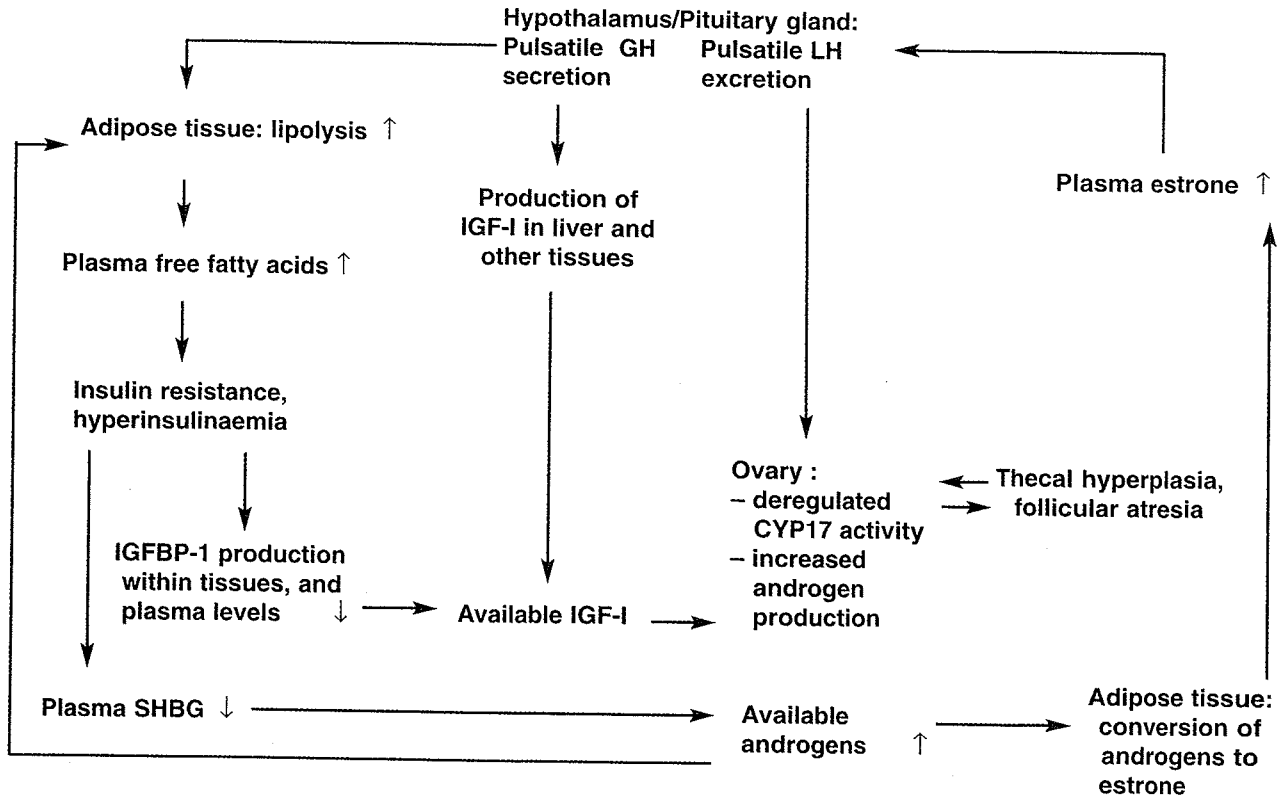

Figure 29 Role of hyperinsulinaemia in the development of functional ovarian hyperandrogenism (polycystic ovary syndrome, PCOS). Modifed from Kaaks (1996)

In premenopausal women with PCOS, obesity and chronic hyperinsulinaemia lead to markedly increased ovarian production and plasma levels of Δ4-androstenedione and testosterone (Burghen *et al.*, 1980; Chang *et al.*,1983; Pasquali *et al.*, 1983; Shoupe *et al.*, 1983; Evans *et al.*, 1988; Wajchenberg *et al.*, 1988; Lanzone *et al.*, 1990; Holte *et al.*, 1994; Pasquali *et al.*, 1994; Ehrmann *et al.*, 1995). A very high proportion of women with PCOS are obese, and insulin resistance appears to play a central role in the development of the syndrome (Ehrmann *et al.*, 1995). In women with PCOS, the degree of obesity (BMI) correlates with the frequency of anovulatory cycles, as indicated by frequency of oligomenorrhoea/amenorrhoea, and in these women is thus also a clear negative determinant of ovarian progesterone production (Kiddy *et al.*, 1992; Robinson *et al.*, 1993). Women with PCOS generally have normal to mildly elevated plasma estrogen levels, which may derive in part from increased aromatization of androgens (mostly to estrone) in adipose tissue. Since SHBG levels are reduced, women with PCOS also have increased levels of bioavailable androgens and estrogens, unbound to SHBG (Lobo *et al.*, 1981; Waldstreicher *et al.*, 1988; Ehrmann *et al.*, 1995; Rosenfield, 1999).

After menopause, ovarian production of estrogens stops and conversion of the androgen precursor androstenedione to estrone in adipose tissue becomes the primary source of estrogens in women (Siiteri, 1987; Azziz, 1989). In postmenopausal women, BMI has been frequently associated with increased levels of total and bioavailable estrogens (Austin *et al.*, 1991; Kaye *et al.*, 1991; Katsouyanni *et al.*, 1991), but not with total or free testosterone (Cauley *et al.*, 1989; Kaye *et al.*, 1991; Newcomb *et al.*, 1995; Turcato *et al.*, 1997). The increase in serum estrone and estradiol is linearly related to the degree of obesity (Kaye *et al.*, 1991).

In men, in contrast to postmenopausal women and premenopausal women with PCOS, obesity and chronic hyperinsulinaemia generally correlate inversely with plasma total testosterone levels (Seidell *et al.*, 1990a; Pasquali *et al.*, 1991, 1995; Haffner *et al.*, 1993b, 1994b; Vermeulen *et al.*, 1993, 1996;

Giagulli *et al.*, 1994; Tibblin *et al.*, 1996), and do not lead to any noticeable increase but generally rather a decrease in bioavailable testosterone unbound to SHBG (Strain *et al.*, 1982; Seidell *et al.*, 1990a; Zumoff *et al.*, 1990; Pasquali *et al.*, 1991; Haffner *et al.*, 1993b, 1994b, 1997; Vermeulen *et al.*, 1993; Andersson *et al.*, 1994; Giagulli *et al.*, 1994; Field *et al.*, 1994; Tchernof *et al.*, 1995; Vermeulen, 1996). The generally accepted explanation for this is that free testosterone causes long-loop negative feedback inhibition of pituitary secretion of luteinizing hormone (LH). Thus, decreases in SHBG are accompanied by a decrease in pituitary LH secretion and hence by a decrease in testicular androgen production, while bioavailable testosterone remains approximately constant. In severely obese men, however, not only are total testosterone levels decreased, but also bioavailable testosterone levels are lower, due to additional mechanisms that are less well understood (Veldhuis *et al.*, 1992). Δ4-Androstenedione levels show a negative correlation with BMI in men (Field *et al.*, 1994; Tchernof *et al.*, 1995). Many studies have found a positive relationship between obesity and serum estrogens in males (Brind *et al.*, 1990; Tchernof *et al.*, 1995; Soygür *et al.*, 1996; Hsieh *et al.*, 1998), although some studies show no obesity-related association (Haffner *et al.*, 1993b, 1994b), whereas such an association has not been reported for women (Kirschner *et al.*, 1990; Austin *et al.*, 1991; De Pergola *et al.*, 1994; Bernasconi *et al.*, 1996).

A few intervention studies have been conducted to examine the effects of weight loss on serum testosterone levels. In hyperandrogenic women, weight reduction leads to decreased total and free testosterone concentrations (Pasquali *et al.*, 1989; Guzick *et al.*, 1994; Leenen *et al.*, 1994; Strain *et al.*, 1994; Crave *et al.*, 1995b). A similar response has been found in adolescent girls (Wabitsch *et al.*, 1995), among whom the reduction of free testosterone was more significant in those with initially more abdominal-type obesity. There are very few recent studies on effects of weight reduction on testosterone in men. Earlier data showed that serum total and free testosterone concentrations increased after weight reduction (Stanik *et al.*, 1981; Strain *et al.*, 1988). The increase may be proportional to the magnitude of weight loss (Strain *et al.*, 1988). In contrast, Leenen *et al.* (1994) did not find any change in total or free testosterone concentration in obese men and women, despite a 13.5-kg weight loss. A more detailed description of the effects of weight loss on endogenous hormones in human intervention studies is presented later in this chapter.

Serum estradiol concentration has been reported to decrease in obese men (Leenen *et al.*, 1994) and in obese adolescent girls (Wabitsch *et al.*, 1995) during successful weight reduction, while no change in levels in postmenopausal women was reported by Turcato *et al.* (1997). The effect of weight reduction on hormonal status and the regularity of menstrual cycles are dependent on the baseline hormonal profile and on the magnitude of weight loss. Although large weight losses (>20% of body weight) and reduced estradiol concentration may induce amenorrhoea, weight reduction in massively obese women, with menstrual disturbances caused by hyperandrogenicity, will normalize the menstrual cycle (Pasquali *et al.*, 1989). In this study, the change in fasting estradiol concentration in plasma was inversely related to change in WHR.

Plasma androgen levels of women with PCOS can be reduced to approximately normal levels by weight loss (Kopelman *et al.*, 1981; Bates & Whitworth,1982; Kiddy *et al.*, 1989; Pasquali *et al.*, 1997b) or by insulin-lowering drugs (Ehrmann, 1999; Pugeat & Ducluzeau, 1999).

Girls participating in vigorous sports such as ballet dancing and running frequently experience primary and secondary amenorrhoea, delayed menarche and more irregular cycles, compared with non-athlete girls (Frisch *et al.*, 1980, 1981; Fogelholm *et al.*, 1996b; Fogelholm & Hiilloskorpi, 1999). The late menarcheal age may be caused by intense physical activity and/or dietary restriction, but selection bias (girls with early menarche drop out) cannot be ruled out. Moreover, the low BMI of athletic girls may also contribute to menstrual irregularities (Maclure *et al.*, 1991; Petridou *et al.*, 1996). Late menarche is associated with a prolonged period of anovulatory cycles after menarche and lower estradiol concentrations (Apter, 1996), which is reflected by menstrual irregularities even after puberty (Fogelholm *et al.*, 1996b). A cross-sectional study of 174 girls aged 14–17 years found that those who expended 2500 kJ (600 kcal) or more energy per week (described by the authors as comparable to two or more hours per week in activities such as aerobic exercise classes, swimming, jogging or tennis) were two to three times more likely than less active girls to have anovulatory menstrual cycles (Bernstein *et al.*, 1987).

High physical activity measured by self-reporting and by movement monitors has been found to be associated with decreased serum concentrations of estradiol, estrone and androgens in postmenopausal women (Cauley *et al.*, 1989). This significant association persisted after adjustment for BMI.

Cross-sectional evaluations of baseline testosterone levels have given somewhat conflicting results. Several studies have reported that high physical activity is associated with lower total and free fasting serum testosterone concentrations in men (Arce *et al.*, 1993; De Souza *et al.*, 1994; Tikkanen *et al.*, 1998), but others found no difference in total or free testosterone concentration between male athletes and controls (Bagatell & Bremner, 1990; Tegelman *et*

al., 1990) or between non-athletes with different levels of recreational exercise (Handa *et al.*, 1997). Moreover, serum testosterone levels at rest may be higher in aged male runners compared with controls of similar age (Hurel *et al.*, 1999).

Exercise-induced increases in estradiol and metabolites have been found in adult women (de Crée *et al.*, 1997a) and men (Gray *et al.*, 1993). In contrast, Montagnani *et al.* (1992) found decreased estradiol levels, together with increased metabolic clearance rate, after a 2-h treadmill run in premenopausal women. It should be noted, however, that any hormonal response following an exercise session is difficult to interpret, because of wide between-study variation in, for instance, subjects' age, body composition and training status, mode, intensity and duration of exercise, dietary status and phase of menstrual cycle.

The increase in testosterone levels during an acute exercise session in men is well documented (Jürimäe *et al.*, 1990; Zmuda *et al.*, 1996; Fahrner & Hackney, 1998; Häkkinen *et al.*, 2000). The response in women is less clear (Häkkinen *et al.*, 2000). However, the increase is very short-lasting and baseline levels are reached within 1–2 hours (Zmuda *et al.*, 1996). If the exercise session has been strenuous, serum testosterone concentration in men may even drop below pre-exercise levels (Kraemer *et al.*, 1998).

A high-intensity exercise intervention in 28 untrained college women with normal ovulation and luteal adequacy resulted in reversible abnormal luteal function in two thirds and loss of LH surge in over half of the subjects (Bullen *et al.*, 1985). The most marked disturbances were observed during the periods of most intense training and among those women who had been randomized to a weight loss (vs weight maintenance) group.

Indirect evidence also supports the view that among athletes estrogen metabolism is affected more by training intensity than by volume: the prevalence of menstrual disorders is higher in endurance than in aesthetic (gymnastics, ballet, etc.) athletes, despite clearly more weekly training hours in the latter group (Fogelholm & Hiilloskorpi, 1999). Interventions in previously untrained subjects have mostly shown unchanged testosterone levels after increased physical activity in men (Oleshansky *et al.*, 1990; Houmard *et al.*, 1994) and postmenopausal women (Häkkinen *et al.*, 2000).

Dehydroepiandrosterone and its sulfate

Data on other sex hormones are scarce. DHEA and DHEAS concentrations in obese women are lower than those of normal-weight controls (De Pergola *et al.*, 1994) or similar (Azziz *et al.*, 1994; Bernasconi *et al.*, 1996; Denti *et al.*, 1997; Ivandic *et al.*, 1998). Subnormal concentrations of DHEA (Field *et al.*, 1994) and DHEAS (Field *et al.*, 1994; Tchernof *et al.*, 1995; Hsieh *et al.*, 1998) have also been observed in men, but not consistently (Denti *et al.*, 1997). In women, a high DHEAS/free testosterone ratio is a positive predictor of lower fasting and postprandial insulin concentrations (Ivandic *et al.*, 1998).

DHEAS levels have been reported to be inversely related to WHR in some studies (Haffner *et al.*, 1993b, 1994b; De Pergola *et al.*, 1994) but not in others (Ivandic *et al.*, 1998; Kirschner *et al.*, 1990; Denti *et al.*, 1997). Moreover, the free testosterone/DHEAS ratio is more closely (positively) related to visceral adipose tissue than to BMI (De Pergola *et al.*, 1994).

Leenen *et al.* (1994) found increased DHEAS levels after weight reduction, but Pasquali *et al.* (1989) reported that they were unchanged.

De Souza *et al.* (1994) did not find any difference between athletes' and controls' DHEAS concentrations, and baseline DHEAS levels do not seem to be affected by long-term exercise training (Houmard *et al.*, 1994; Häkkinen *et al.*, 2000).

Other hormones

Obesity seems to have very little effect on prolactin metabolism. Cross-sectional comparisons have not revealed any difference in serum prolactin concentration between obese and normal-weight subjects (Coiro *et al.*, 1990; Scaglione *et al.*, 1991). In agreement with these cross-sectional findings, serum prolactin concentration was also found to remain unchanged after weight reduction (Hainer *et al.*, 1992).

Physical exercise acutely increases serum prolactin concentration in both males and females (Jürimäe *et al.*, 1990; Oleshansky *et al.*, 1990; Gray *et al.*, 1993). The increase may be more than 200% above resting levels. However, Arce *et al.* (1993) and De Souza *et al.* (1994) reported no difference in baseline prolactin level between well trained male athletes and untrained controls.

Immune function

The immune system has been hypothesized to modulate the likelihood of tumour formation by inhibiting cell growth and countering the action of tumour growth promoters (Shephard & Shek, 1998). The most often studied immunological outcomes are numeration of immune cells and tests of their functional capacity (Stallone, 1994). Neutrophil, monocyte and total lymphocyte counts, as well as phenotypic analyses of lymphocyte and immunoglobulin populations, are common measures. Phenotypic analysis of lymphocytes may give more information than the total count. However, the interpretation of the results is complicated by the increased number of variables. Lymphocyte activation by mitogens *in vitro* provides specific information about cellular functions. Some studies have used less specific functional tests, such as measurement of delayed-type hypersensitivity.

Several studies have shown impaired immune function in obese children (Chandra & Kutty, 1980) and adults

(Weber *et al.*, 1986; Tanaka *et al.*, 1993), but results have been inconsistent. Moriguchi *et al.* (1995) reported impaired immune function (natural killer (NK) cell cytotoxic activity, mitogenic stimulation of lymphocytes) in obese elderly subjects (60–69 years), whereas immune function in younger obese subjects tended to be better than in normal-weight controls. Nieman *et al.* (1996) also found indications of improved immunity in obese subjects.

The acute effects of weight reduction on immune function are more straightforward: most indices show impaired immune status. Several papers report decreased leukocyte, neutrophil, monocyte, NK cell counts and/or immunoglobulin concentration after weight reduction (mean 7–32 kg) by very low-energy diet or other protein-modified fasts (Merritt *et al.*, 1980; Field *et al.*, 1991; Nieman *et al.*, 1996) or after a low-energy diet (Kelley *et al.*, 1994). Decreased delayed-type hypersensitivity (Stallone *et al.*, 1994), mitogen-induced lymphocyte proliferation (Field *et al.*, 1991; Tanaka *et al.*, 1993; Nieman *et al.*, 1996) and bacterial killing and chemotaxis of granulocytes (McMurray *et al.*, 1990) are other findings of impaired immune function during weight reduction. It is not possible to tell whether the observed changes are caused by inadequate intake of nutrients or by negative energy balance and weight reduction *per se*.

Two studies examined the effects of additional exercise training during weight reduction by dietary techniques. Nieman *et al.* (1998) reported that moderate aerobic exercise training did not affect immune function during weight reduction, but the number of days with symptoms of upper respiratory tract infections was lower in exercising than in diet-only subjects. In another study (Scanga *et al.*, 1998), exercise training prevented a weight-reduction-induced decrease in NK cell cytotoxicity. However, leukocyte and lymphocyte counts decreased during weight reduction, with or without added exercise training.

Decreased immune cell counts have also been observed in anorexia nervosa (Marcos *et al.*, 1997a) and bulimia nervosa (Marcos *et al.*, 1997b) patients. Among bulimic patients, those with low weight had the most deteriorated status. A decrease in NK cell count was the most consistent finding in both studies. Partial weight regain in anorexia nervosa patients was followed by a partial recovery in the immunological indices, except for NK cell count, which remained low (Marcos *et al.*, 1997a).

Several potential mechanisms could explain, at least partly, the observed associations between obesity and immune function (Kumari & Chandra, 1993; Stallone, 1994). An excess dietary intake of certain lipids may impair immune status. Both inadequate and very high intakes of several micronutrients (e.g., vitamins A and E, carotenoids, iron, zinc and selenium) may have negative effects on immune status. Ketosis, an almost inevitable consequence of a very low-energy diet, may also inhibit immune cell function by inhibiting glucose uptake and utilization.

A session of physical activity has marked acute effects on the immune system. Several types of immune cell have been observed to increase acutely in response to exercise, including neutrophils, monocytes, eosinophils and total lymphocytes (Eliakim *et al.*, 1997; Gabriel & Kindermann, 1997; Robson *et al.*, 1999; Moldoveanu *et al.*, 2000), but the levels may remain unchanged after eccentric exercise (Miles *et al.*, 1999). After a strenuous exercise session, the increased levels of indicators of immune function may still be observable after 24 h (Eliakim *et al.*, 1997; Gabriel & Kindermann, 1997).

The number of NK cells increases sharply during high-intensity activity, but decreases during very prolonged moderate-intensity activity (Tvede *et al.*, 1993; Eliakim *et al.*, 1997; Gabriel & Kindermann, 1997). After exercise, NK cell number decreases more rapidly subpopulation (Eliakim *et al.*, 1997; Gabriel & Kindermann, 1997). A suggested reason for post-exercise suppression of NK cell activity is inhibition by prostaglandins released by monocytes (Tvede *et al.*, 1993). NK cell count does not seem to be affected by eccentric exercise (Miles *et al.*, 1999).

Pedersen *et al.* (2000) recently reviewed the effects of an acute bout of exercise on cytokine production. The plasma levels of various cytokines (TNF-α, IL-1β, IL-6) increase during intense exercise. The increase of IL-6 is proportionally much greater than that of other cytokines. In a systematic review on mucosal immunity in élite athletes, Gleeson (2000) identified 25 studies on the effects of an acute exercise bout on salivary concentration of immunoglobulin (Ig)A. Sixteen studies showed decreased levels and only two reported increased IgA after exercise. IgA concentration in saliva returns to basal levels within 1 h after physical activity.

Very few functional tests have been used to study immune response to physical activity. However, Bruunsgaard *et al.* (1997) studied cell-mediated immunity evaluated by skin tests in 22 males after intense, long-duration exercise (triathlon). The results indicated impaired immunity in the first few days after the exercise.

Studies on immune function during long-term training have yielded somewhat inconsistent results. Many have found depressed immune function following intense physical training, using NK cell count, neutrophil oxidative capacity, T-cell responses *in vitro* or saliva IgA concentration as a marker (Pyne *et al.*, 1995; Kramer *et al.*, 1997; Gleeson, 2000). Nine out of 12 studies identified in the review by Gleeson (2000) reported decreased salivary IgA concentration in athletes after long-term training, indicating cumulative mucosal

immune suppression over training periods. In contrast, moderate exercise training does not seem to have any negative effect on IgA level (Mackinnon, 2000).

In contrast to the above studies, others have suggested that physical activity may be related to improved immune function. Kumae *et al.* (1999) found increased lymphocyte and eosinophil counts, but decreased neutrophil generation of reactive oxidative species in runners. Moderate exercise training has been shown to lead to increased immunoglobulin levels (Nehlsen-Cannarella *et al.*, 1991), elevated NK activity or cell count (Watson *et al.*, 1986; Nieman *et al.*, 1990; Rhind *et al.*, 1994, 1996; Hoffman-Goetz *et al.*, 1998), changes in lymphocyte subsets (LaPerriere *et al.*, 1994; Host *et al.*, 1995), IL-2 production and IL-2 receptor expression (Rhind *et al.*, 1994, 1996) and immunoglobulin levels (Nehlsen-Cannarella *et al.*, 1991).

Nieman *et al.* (1993) conducted a randomized controlled trial (walking vs calisthenics) among 32 sedentary, elderly Caucasian women (67–85 years). Although the exercise training programme (5 × 30–40 min/wk for 12 weeks) resulted in no significant improvement in either NK cell or T-lymphocyte function, the incidence of upper respiratory tract infections was significantly lower in the walking group (21%) than in the calisthenics group (50%), and was lowest in a comparison group of highly trained elderly people (8%). These results are consistent with those from a trial in younger obese women (mean age 35 years) in which exercise subjects (45 min of walking, 5 d/wk) experienced half the number of days with symptoms of upper respiratory tract infection during a 15-week period compared with a sedentary control group (5.1 vs 10.8, $p < 0.05$) (Nieman *et al.*, 1990). Nieman *et al.* (1995) also reported higher NK cell cytotoxic activity in marathon runners compared with sedentary controls. In contrast, extremely heavy exertion (such as a 90 km run) has been shown to increase the incidence of upper respiratory tract infections (Peters, 1997).

In a recent review, Mackinnon (2000) presented a summary of resting values of immune function variables in athletes, compared with those of non-athletes or with clinical norms. Many indices (leukocyte, granulocyte and lymphocyte number, serum specific antibody) were similar in athletes and controls. NK cell number and cytotoxic activity, and lymphocyte activation and proliferation, were normal or higher in athletes. In contrast, neutrophil function and immunoglobulin concentration in serum and mucosa were clearly suboptimal in athletes. Hence, compared with studies on moderate training (Nieman *et al.*, 1990; Nehlsen-Cannarella *et al.*, 1991), athletic training seems to have more deleterious effects on immune function.

In summary, immune responses to an acute exercise bout include increased immune cell counts and cytokine production, but decreased saliva IgA concentration, immediately after intense activity. After strenuous exertion, the immune cell counts decrease rapidly and may reach levels that are clearly below pre-exercise values 1–3 h after cessation. This has been referred to as an "open window of decreased host protection" (Nieman, 1997). Most immune variables reach normal or slightly elevated levels 24 h after exercise. Responses are less clear after moderate-intensity and short-duration activity. Cross-sectional comparisons between trained and untrained subjects have yielded inconclusive results (Nieman, 1997; Jonsdottir, 2000). However, many authors hold the view that moderate physical activity has a positive effect on the immune system (Peters, 1997; Shephard & Shek, 1998; Jonsdottir, 2000). The inconsistencies in study results described above may be due to the use of different immune measures, differences in exercise regimen, small sample sizes with insufficient statistical power to detect changes, characteristics of the participants, or effects of unmeasured confounding factors, such as nutritional factors, smoking or alcohol intake and stress levels. It is unclear to what extent the results in young adults or athletes pertain to exercise in the elderly.

Intervention trials

Weight reduction

Table 21 summarizes many weight reduction studies that have been carried out with different intervention strategies in obese patients, pre- and postmenopausal women, men, children and adolescents.

The overall picture is that, with weight loss, insulin resistance decreases, and basal and postprandial glucose and insulin levels are similarly reduced. Reduced fasting glucose and insulin levels have been consistently observed, whether weight was lost with a low-energy diet (usually 4200–6300 kJ (1000 to 1500 kcal)/day) or a very low-energy diet (1700–3400 kJ (400 to 800 kcal)/day), with or without associated physical activity or pharmacological treatment. Glucose and insulin areas after glucose tolerance test and blood lipid patterns improved as well. Plasma levels of SHBG were also consistently shown to increase with weight reduction, while the effects on sex steroid hormones were less clear-cut and were different in men and women. It is not clear, however, if the improvement in insulin sensitivity which has been demonstrated in short-term studies is sustained over longer periods. On average, it appears that a 15-week diet or diet-and-exercise programme can achieve an approximately 11-kg weight loss, with a 6–8-kg maintained loss after one year. Few studies appear to have been continued for longer than a year and those few show generally disappointing results (reviewed by Mann, 2000). It does not seem necessary, however, to achieve the ideal body weight to

improve the metabolic profile – in most instances a 5–10% weight reduction is sufficient to induce a clinically beneficial effect (Weinstock *et al.*, 1998).

Some of the randomized controlled trials and the largest uncontrolled intervention studies are presented below in some detail, but several other trials are summarized in Table 21.

Premenopausal women. Fujioka *et al.* (1991) investigated 40 premenopausal Japanese obese women, aged 38 years, with mean BMI 35 kg/m^2 at recruitment, in whom substantial weight reduction was obtained by means of a low-energy diet. In 14 women with predominantly visceral fat tissue (as determined by computed tomography scan), BMI decreased from 34.3 to 29.4 kg/m^2 and in 26 women with predominantly subcutaneous adipose tissue, BMI decreased from 36.0 to 30.9 kg/m^2. Fasting plasma glucose and insulin levels, plasma glucose and insulin areas after an oral glucose tolerance test and total cholesterol and triglycerides levels decreased significantly. The changes in plasma glucose area and in triglyceride levels were significantly correlated with changes in the ratio of visceral to subcutaneous fat volume ($r = 0.307$ and 0.486, respectively).

Guzick *et al.* (1994) randomized 12 obese, hyperandrogenic, anovulatory women to a 12-week weight loss programme or a 12-week 'waiting list': in the intervention group, body weight decreased from 108.0 ± 5.3 to 91.8 ± 6.0 kg, and SHBG increased from 0.60 ± 0.09 to 0.73 ± 0.09 mg/dL. Crave *et al.* (1995b) randomized 24 obese (BMI > 25 kg/m^2) hirsute patients with high fasting insulin and low SHBG levels into a metformin and a placebo group, and both were treated with a low-energy diet (6300 kJ (1500 kcal)/day with 30% fat) for four months. SHBG increased significantly in both groups (from 19.1 ± 1.9 to 26.0 ± 3.3 nmol/L in the placebo group and from 17.6 ± 1.6 to 21.6 ± 2.1 in the metformin group). Both studies showed decreases in free testosterone corresponding to the increase in SHBG and a decline (though in the study by Guzick not statistically significant) in fasting insulin after the dietary intervention. Androstenedione decreased and DHEAS increased (Crave *et al.*, 1995b), but there was no reduction in serum total testosterone. Nevertheless, several studies showed that energy restriction reduced total testosterone level, especially in obese, infertile hyperandrogenic women with polycystic ovaries. The results on total serum estradiol were inconsistent, with some studies showing a significant reduction and some others no effect.

Weinstock *et al.* (1998) randomized 45 obese non-diabetic women (aged 43.3 ± 1.1 years; mean baseline weight 96.9 ± 2.2 kg and BMI 35.9 ± 0.9 kg/m^2) to one of three treatment groups: diet alone, diet and aerobic training, diet and strength training. All subjects received the same 48-week group behaviour modification programme and diet (3900 kJ (925 kcal)/d for the first 16 weeks, 6300 (1500 kcal)/d thereafter). During weeks 48 to 96, subjects were unsupervised. Subjects on all three treatments achieved a mean weight loss of 13.8 kg by week 16, which was associated with decreased fasting insulin levels (from 15.4 ± 1.0 to 10.6 ± 0.6 mU/L) and insulin area after an oral glucose tolerance test (61.8% of baseline), without significant differences among groups and without further improvement with further unsupervised diet. Fasting glucose and the area under the glycaemic curve after an oral glucose tolerance test decreased slightly after weight loss, but this change was not significant. Only 22 out of the initial 45 subjects were studied at week 96. They maintained a loss of approximately 10% of the initial weight (–9.9 kg), but insulin levels had returned to pretreatment levels.

Postmenopausal women. Svendsen *et al.* (1995) carried out a clinical intervention study on 98 healthy, overweight, postmenopausal women aged 49–58 years, with BMI 25–42 kg/m^2, who were given a 4200 kJ (1000 kcal) diet daily for three months. Increased SHBG was significantly correlated with reduction in weight and loss of fat determined by total body dual-energy X-ray absorptiometry (DXA) scanner ($r = -0.4$ to -0.5, $p < 0.01$) and with reduction in waist circumference, visceral adipose tissue and WHR ($r = -0.3$ to -0.4, $p < 0.01$). There was no reduction in serum levels of total testosterone or estradiol.

Kasim-Karakas *et al.* (2000) modified the diet of 64 healthy postmenopausal women with a mean age of 61 ± 11 years and a mean baseline weight of 75 ± 2.4 kg. For the first four months, participants followed a controlled-energy diet with stepwise reduction of fat intake from 35% to 25% to 15% of energy. They were then requested to follow a self-selected 15% fat diet *ad libitum* for eight months. Mean carbohydrate consumption increased from 200 g/day at baseline to 377 g/day at four months and 256 g/day at 12 months. The actual food composition of the diet was not published. During the *ad libitum* diet, the mean weight loss was 4.6 ± 0.5 kg, fasting plasma glucose decreased significantly (down to 4.77 ± 0.11 mmol/L from 5.44 ± 0.11 at baseline and 5.16 ± 0.11 at four months), insulin decreased non-significantly, triglycerides increased during the euenergetic treatment and returned to baseline values after the *ad libitum* period, total, high- and low-density lipoprotein (HDL and LDL) cholesterol decreased in the first four months but LDL returned to baseline values after the *ad libitum* period. Similar weight loss with an *ad libitum* low-fat diet without increasing triacylglycerolaemia was observed in other studies (Berrino *et al.*, 2001; Schaefer *et al.*, 1995).

Studies in NIDDM patients. Wing *et al.* (1991) randomly assigned 36 type-II diabetic subjects (10 men and 26 women,

age 35–70 years), 30% or more above ideal body weight, either to a low-energy diet for 20 weeks or to the same programme that included, however, an eight-week period of very low-energy diet (1700 kJ (400 kcal) of lean meat, fish and fowl). Both diets were associated with behaviour therapy and an exercise programme. 33 subjects completed the 20-week programme and complied with the one-year follow-up. The very low-energy group had greater weight loss at week 20, but weight losses from pretreatment to one year were similar (from 102.1 to 93.5 kg and from 104.5 to 97.7 kg on average). The very low-energy diet produced a greater decrease in fasting glucose at the end of the 20-week programme (–6.5 mmol/L vs –3.5, $p = 0.035$) and at one year (–3.8 vs +0.7 mmol/L, $p = 0.001$) and greater long-term reduction of glycosylated haemoglobin. The very low-energy group, however, had a greater rise in insulin during the oral glucose tolerance test carried out at 20 weeks, suggesting to the authors that the improved glycaemic control could be due to an increase in insulin secretion. The change in average fasting insulin from baseline to 20 weeks to one year was from 141 to 120 to 133 pmol/L in the low-energy group, and from 163 to 104 to 205 pmol/L in the very low-energy group. A similar biphasic effect on plasma insulin was previously observed by Stanik and Marcus (1980), who placed seven severely hyperglycaemic obese patients (men and postmenopausal women) on severe energy restriction for 4–12 weeks. On entry, mean fasting plasma glucose level was 326 ± 23 mg/dL. The insulin response to oral glucose was completely flat. After initiating caloric restriction, fasting plasma glucose rapidly fell, reaching 150 ± 21 mg/dL by two weeks, and remained low throughout the diet period. At restudy, improved oral glucose tolerance was accompanied by significant increases in the insulin secretory responses to both glucose and tolbutamide. These results support the concept that control of plasma glucose concentration allows recovery of insulin secretion. The degree of weight loss necessary to achieve this effect was modest.

Treatment of NIDDM patients (10 males aged 40–70 years, WHR > 0.9 and mean BMI 26 kg/m^2) with dexfenfluramine (and low-energy diet) for three months resulted in a significant decrease in visceral (from 484 ± 230 to 333 ± 72 cm^2) rather than subcutaneous adipose tissue. This specific decrease in visceral adipose tissue was accompanied by a remarkable increase in insulin sensitivity, as assessed by the minimal model technique (from 0.29 ± 0.13 to 0.54 ± 0.21 min^{-1} mU/L, $p = 0.01$), significantly decreased levels of C-peptide (from 0.77 ± 0.24 to 0.58 ± 0.15 mmol/L, $p = 0.002$), total cholesterol and triglycerides ($p < 0.001$ and $p = 0.021$) and non-significantly decreased fasting glucose and glycosylated haemoglobin (Marks *et al.*, 1996).

Further studies showing improved glucose control in diabetic patients are cited in Chapter 6.

Studies in men and both sexes together. Stanik *et al.* (1981) investigated the effects of weight reduction on reproductive hormones in 24 moderately obese men, 18–108% above ideal body weight. Serum estrone, estradiol, testosterone, percentage free testosterone, SHBG binding capacity, and, in nine subjects, androstenedione were measured serially before and during an outpatient supplemented fasting programme (1300 kJ (320 kcal)/day) for 8–20 weeks. At the baseline, mean estrone was elevated to 100 ± 7 pg/mL (normal, 30–60 pg/mL). Estradiol was slightly elevated to 36 ± 3 pg/mL (normal, 8–35 pg/mL). The mean testosterone level of 400 ± 20 ng/dL was at the lower end of the normal range (400–1000 ng/dL) but the mean % free testosterone was elevated to 4.1 ± 0.2% (normal 1.6–3%). The calculated free testosterone level was normal. The mean SHBG binding capacity was 0.99 ± 0.05 µg dihydrotestosterone bound/dL (normal, 1.0–1.8 µg/dL). The mean androstenedione level of 52 ± 5.8 ng/dL was normal. These data were consistent with previous findings in much heavier men. Weight loss (mean, 19.5 kg) after eight weeks was associated with normalization of all the measured parameters: mean estrone decreased to 48 ± 23 pg/mL ($p < 0.01$), estradiol to 28 ± 2.1 pg/mL ($p < 0.05$), testosterone increased to 536 ± 35 pg/dL and % free testosterone fell to 3.2 ± 0.2% (both $p < 0.01$). Data on 16 men remaining on the programme for 16 or 20 weeks showed a continued fall of estrogens and stabilization of testosterone and % free testosterone. However, unlike the findings of increased SHBG binding capacity with weight loss in obese women, SHBG did not change significantly over the entire time period.

Tymchuk *et al.* (1998) measured the levels of insulin, SHBG, prostate-specific antigen (PSA) and serum lipids in 27 obese men (mean age 57± 2.6 years) undergoing a three-week low-fat (< 10% of calories) high-fibre and high complex carbohydrate diet and exercise programme. BMI decreased from 35 ± 1.9 to 33.4 ± 1.8 kg/m^2. Insulin decreased from 222 ± 30 to 126 ± 21 pmol/L and SHBG increased from 18 ± 2 to 25 ± 3 nmol/L. PSA decreased but not significantly (all the three men with slightly elevated levels showed a decrease). Triglycerides and total and LDL and HDL cholesterol decreased significantly (average 41, 49, 37, and 5.3 mg/dL, respectively), as did the total/HDL cholesterol ratio (all $p \leq 0.01$). Sex steroids were not measured, but in a previous study on 21 men, the same intervention for 26 days decreased serum estradiol levels from 47.2 ± 4.6 to 23.8 ± 2.5 pg/mL whereas serum testosterone levels were unchanged (5.1 ± 0.3 versus 5.1 ± 0.2 ng/mL). Total serum cholesterol levels decreased from 229 ± 9 to 181 ± 7

Table 21. Hormonal and metabolic effects in weight reduction trials on obese subjects

(a) Studies on premenopausal women

Reference	Mean age or range	No.	Type (daily energy) and duration of intervention	Initial BMI or % IBW*, weight	Weight change
Low-energy diet					
Bates & Whitworth, 1982	NR	7[b]	Unspecified caloric restriction	141*	–15%
Grenman *et al.*, 1986	35	25	1200 kcal, 12 mo	43, 121	–11%
Pasquali *et al.*, 1989	22	20	1000–1500 kcal, 6–12 mo	32, 86	–11%
Leenen *et al.*, 1994	39	33	4.2 MJ deficit, 3 mo	31, 87	–14%
Slabber *et al.*, 1994[d]	35	30	Low glycaemic index vs	35, 94	–10%
			balanced diet, both 1000–1200 kcal, 12 wk	35, 97	–7%
Crave *et al.*, 1995b	NR	24[e]	1500 kcal, 4 mo	34, 87.5	–4%
Turcato *et al.*, 1997	34	26	1286 kJ, 4 wk	38, 101	–8%
Weinstock *et al.*, 1998	43	45	925–1500 kcal ± EX, 58 wk	36, 97	–14%
Very low-energy diet					
Harlass *et al.*, 1984	NR	6[e]	500 kcal 4–6 mo	>30, 103	–11%
Kiddy *et al.*, 1989	NR	5[f]	330 kcal, 4 wk	36	–5.2 kg
Fujioka *et al.*, 1991	38	40[f]	800 kcal, 8 wk	35, 86	–14%
Wing *et al.*, 1992[g]	38	101	1000–1500 kcal + EX, 20 wk	31, 83	–8%
Hamilton-Fairley *et al.*, 1993	NR	6[b,e]	350 kcal, 4 wk	34	–7%
Zamboni *et al.*, 1993	39	16	1286 kJ x 2 wk + 4200 kJ x 14 wk	38, 104	–15%
Guzick *et al.*, 1994[g]	32	12	400–1200 kcal + EX, 12 wk	178*, 108	–15%
Holte *et al.*, 1995[h]	NR	13[f]	1200 kcal, up to stable weight	32, 89	–14%
Jacubowicz & Nestler, 1997	30	11	1000–1200 kcal, 8 wk	32	–7% (BMI)
Jacubowicz & Nestler, 1997	29	12[f]	1000–1200 kcal, 8 wk	32	–7% (BMI)
De Pergola *et al.*, 1998	32	21	318 kcal, 3 wk	39	

(b) Studies on postmenopausal women

Reference	Mean age or range	No.	Type (daily energy) and duration of intervention	Initial BMI or % IBW*, weight	Weight change
Low-energy diet					
Svendsen *et al.*, 1995	49–58	98	4.2 MJ ± EX, 3 mo	> 25, 78	–13%
Kasmin-Karakas *et al.*, 2000	61	64	15% fat, 1 y	28, 75	–8%
Very low-energy diet					
O'Dea *et al.*, 1979	50–63	12	Supplemented fast, 12–17 wk	124–193*, 105	–23%
Turcato *et al.*, 1997	58	15	1286 kJ, 4 wk	35, 88	–6%

fG	fI	IGF	BP[a]	Ga	Ia	CP	IS	SH	E1	E2	T	fT	A	D	tri	chl	Other relevant effects
											↓		↓				
								(↑)[c]	↑	=	=		↑				C↓, PRL↓
					↓				(↓)	(↓)	↓	=	(↓)	(↓)			LH↓, P↓
	↓							↑	=	↓	↓	↓	↓	↑			fE2↓
=	↓					=											
=	(↓)					↑											
=	↓							↑			=	↓	↓	↑	(↓)	(↓)	
↓	↓							↑	(↑)		(↑)	↓	(↓)				LH↓ C↓
(↓)	↓[c]			(↓)	↓												
								↑			↓						LH↓
	↓	↓	1↑					↑			=	↓					
↓	↓			↓	↓										↓	↓	
↑				(↓)											=	↓	
			1=	=	↓			↑									
↓	↓			↓	↓										↓	↓	
(↓)	(↓)							↑			(↓)	↓					
=	↓	=		(↓)	↓	(↓)	↓	↑			↓		=	=			LH =
=	↓							↑			=	=	=	↑			LH =
=	↓							↑			↓	↓	=	=			17αOHP↓, LH =
	↓	↓	3↑														

fG	Hb	fI	SH	E2	T	fT	A	D	tri	chl	Other relevant effects
			↑	=	=		=				
↓	=	(↓)							=	↓	FFA ↓
			↑	↓	=	↓					LH ↑
↓		=	(↑)		=	(↑)	(↓)				LH ↑, C↓

Table 21 (contd)

(c) Studies in children and adolescents

Reference	Sex	Mean age or range	No.	Type (daily energy) and duration of intervention	Initial BMI or % IBW*, weight	Weight change
Pintor *et al.*, 1980	Girls	7–11	12	1000 kcal until IBW	175–220*	NR
Knip & Nuutinen, 1993	Both	6–16	32	Hypocaloric, 1 y	29, 69	–16%
Wabitsch *et al.*, 1995	Girls	15	92	4321 kJ + EX, 6 wk	31, 87	–10%

(d) Studies on men and both sexes together

Reference	Sex	Mean age or range	No.	Type (daily energy) and duration of intervention	Initial BMI or % IBW*, weight	Weight change
Low-energy diet						
Rosenthal *et al.*, 1985	M	57	21	LF-HF + EX, 26 d	NR, 99	–5%
Strain *et al.*, 1988	M	20–50	11	Individual low-cal diet, 17 mo	167*	26–130 kg
Wing *et al.*, 1992[g]	M	37	101	1000–1500 kcal + EX, 20 wk	31, 96	–10%
Leenen *et al.*, 1994	M	40	37	4.2 MJ deficit, 3 mo	31, 97	–14%
Rasmussen *et al.*, 1994	B	36	60	1200 kcal, 16 wk	34, 96	–8%
Colman *et al.*, 1995[h]	M	60	35	LED, 9 mo	30, 91	–10%
Rasmussen *et al.*, 1995	NR	NR	9	1.6 MJ[i]	39, 111	–27%
Dengel *et al.*, 1996[g]	M	59	47	300–500 kcal deficit ± EX, 10 mo	120–160*, 92	–10%
Torjesen *et al.*, 1997[g]	B	40	219[j]	Low-fat, high fish ± EX, 1 y	29	–9%
Goodpaster *et al.*1999	B		32	800–1200 kcal, 13 wk[k]	35, 100	–13%
Ross *et al.*, 2000a[g]	M	45	52	700 kcal deficit ± EX, 12 wk	31, 96	–8%
Very low-energy diet						
Stanik & Marcus, 1980	B	48–67	7	600 kcal, 4–12 wk	169*, 107	–10%
Stanik *et al.*, 1981	M	30–63	24	320 kcal, 8 wk	154*, 112	–17%
Gama *et al.*, 1990	B	41	7	445 kcal, 3 wk	35, 97	–5%
Tanaka *et al.*, 1990	B	16–29	15[f]	VLED, over 1 wk	34, 87	–13%
Wing *et al.*, 1991[g]	B	51	33[l]	VLED programme, 20 wk	38, 103	–7%
Megia *et al.*, 1993	B	43	20	Fasting or VLED, 29 d	46	–12% (BMI)
Strain *et al.*, 1994	M	NR	17	LF-HF low cal, 6 wk–39 mo	51	–19%
Vermeulen *et al.*, 1996	M	25–62	50	PSMF, 6 wk	41, 124	–12%
Tymchuk *et al.*, 1998	M	57	27	LF-HF + EX, 3 wk	35	–5%
Fogelholm *et al.*, 2000a	M	40	74	VLED programme, 12 mo	34, 92	–14%

fI	CP	SH	E2	T	fT	A	D	tri	Other relevant effects
				=		↓	↓		C =, PRL =, P ↓
↓								↓	
↓	↑	↑	↓	↓	↓	=	↓		fC ↑

fG	Hb	fI	IGF	Ga	Ia	CP	IS	SH	E1	E2	T	fT	A	D	tri	chl	Other relevant effects
=										↓	=				↓	↓	
								↑	(↑)	=	↑	↑					
=				(↓)											↓	↓	
		↓						↑	↑	↓	=	=	↓	↑			fE2↓
↓		↓	↑[c]														GH = , BP-3 =
=		↓		↓	↓												
			↑														GH↑, BP-3 =
=		↓		↓	↓												
↓		↓		↓		↓	↑								↓		
=		↓					↑								↓	↓	Leptin ↓
=		(↓)		=	(↓)		↑										
↓							↑										
								=	↓	↓	↑	%↓	=				
=		↓		=	↓												GH ↑
=		↓															GH ↑
↓	↓	↓[c]													(↓)	(↓)	
↓		↓	↓														GH ↑
		↓						↑									
		↓	↑					↑		↓	↑	=	(↓)	=			GH(↑)
		↓						↑							↓	↓	
		↓															

Table 21 (contd)

(e) Intervention trials with drugs or surgery

Reference	Sex	Mean age or range	No.	Type (daily energy) and duration of intervention	Initial BMI or % IBW*, weight	Weight change
Kopelman *et al.*, 1981	Wp	30	12	Bypass surgery, 1 y	225*, 126	–31%
Poulos *et al.*, 1994	NR	42	50	Bypass surgery, 1 y	45, NR	–29%
Marks *et al.*, 1996	M	40–70	10[l]	Dexfenfluramine, 12 wk	26, 78	–4%
Van Gaal *et al.*, 1998[g]	NR	43	55	600 kcal deficit, EX, ± sibutramine, 6 mo	36	–11 kg
Sjöström *et al.*, 1998[g]	B	45	688	600 kcal deficit ± orlistat, 1 y	36, 99	–10% vs –6%
Davidson *et al.*, 1999[g]	B	44	892	2100–3360 kJ deficit ± orlistat, 2 y	36, 100	–9% vs –6%
James *et al.*, 2000[g]	B	40	467	600 kcal ± sibutramine, 2 y	37, 102	–10% vs –5%

Arrows indicate a statistically significant increase or decrease; arrows in brackets indicate a non-statistically significant increase or decrease greater than 10%; =, no change, IBW, ideal body weight; LED, low-energy diet; VLED, very low-energy diet; LF-HF, low-fat, high complex carbohydrates and fibre; PSMF, protein-sparing modified fast; EX, exercise; y, years; mo, months; wk, weeks; d, days; NR, not reported
Wp, women, premenopausal; M, men; B, both men and women
fG, fasting glucose; Hb, glycosylated haemoglobin; fI, fasting insulin; IGF, insulin-like growth factor-I; BP, IGF-binding protein; Ga, glucose area (or glucose at 2 h) under glucose tolerance test; Ia, insulin area; CP, C peptide; IS, insulin sensitivity; SH, sex-hormone binding globulin; E1, estrone; E2, estradiol; T, testosterone; fT, free testosterone; A, androstenedione; D, dehydroepiandrosterone sulfate; tri, triglycerides; chl, cholesterol; FFA, free fatty acids; GH, growth hormone; C, cortisol; PRL, prolactin; P, progesterone; LH, luteinizing hormone; fC, free cortisol; fE2, free estradiol; 17αOHP, 17α-hydroxyprogesterone

Energy values are given in calories or joules as in the publication cited: 1 kcal = 4.19 kJ

fG	Hb	fI	IGF	CP	IS	SH	E1	E2	T	A	tri	chl
						↑	(↑)	↑	↓	↓		
↓	↓	↓	↑									
(↓)	(↓)			↓	↑						↓	↓
↓		↓									↓	
↓		↓									=	↓
=		↓									=	↓
=		↓		↓							↓	=

[a] IGFBP-1 or IGFBP-3 as indicated
[b] PCOS patients (the study by Kiddy included also six normal-weight women, with the same results; in particular IGF-I decreased significantly in two weeks)
[c] Early decrease followed by an increase to baseline values (or vice versa)
[d] Cross-over trial
[e] Hirsute patients
[f] Japanese subjects
[g] Randomized trial
[h] Controlled trial
[i] The intervention continued up to when body weight was 30% above IBW
[j] All subjects aged 40 years
[k] Ascertainment at 17 weeks
[l] NIDDM patients

mg/dL, whereas triglyceride levels fell from 301 ± 66 to 151 ± 13 mg/dL. HDL cholesterol levels fell from 41 ± 3 to 35 ± 1 mg/dL, whereas the total/HDL cholesterol ratio was unchanged (5.5 ± 0.4 versus 5.1 ± 0.3) (Rosenthal *et al.*, 1985).

Goodpaster *et al.* (1999) examined the relationship between weight loss-induced change in regional adiposity and improvement in insulin sensitivity. 32 obese sedentary women and men completed a four-month weight loss programme and had repeat determinations of body composition (DXA and computed tomography) and insulin sensitivity (euglycaemic insulin infusion). 15 lean men and women served as control subjects. The intervention achieved significant decreases in weight (100.2 ± 2.6 to 85.5 ± 2.1 kg), BMI (34.3 ± 0.6 to 29.3 ± 0.6), total fat mass (36.9 ± 1.5 to 26.1± 1.3 kg), percentage body fat (37.7 ± 1.3 to 31.0 ± 1.5%) and fat-free mass (FFM) (59.2 ± 2.3 to 55.8 ± 2.0 kg). Abdominal subcutaneous and visceral adipose tissue were reduced (494 ± 19 to 357 ± 18 cm^2 and 157 ± 12 to 96 ± 7 cm^2, respectively). Insulin sensitivity improved from 5.9 ± 0.4 to 7.3 ± 0.5 mg × FFM^{-1} × min^{-1}. Rates of insulin-stimulated non-oxidative glucose disposal accounted for the majority of this improvement. Serum levels of leptin, triglycerides, cholesterol and insulin all decreased ($p < 0.01$). After weight loss, insulin sensitivity continued to correlate with generalized and regional adiposity but, with the exception of the percentage decrease in visceral adipose tissue, the magnitude of improvement in insulin sensitivity was not correlated with the various changes in body composition.

Several randomized controlled trials have addressed the issue of adding an aerobic exercise programme to a low-energy diet for weight reduction and improvement of metabolic parameters in obese subjects. Studies with a factorial design have usually shown that a low-energy diet is more effective than aerobic exercise, but the combination of both may improve insulin-linked parameters (Dengel *et al.*, 1996; Torjesen *et al.*, 1997; Ross *et al.*, 2000a).

Studies with drugs. James *et al.* (2000) reported on the efficacy of treatment with sibutramine (an inhibitor of serotonin and adrenalin uptake that increases satiety) for weight maintenance after weight loss in otherwise healthy obese patients of both sexes, aged 17–65 years, with initial BMI between 30 and 45 kg/m^2. 467 patients who succeeded in losing more than 5% of their weight over six months of low-energy diet and sibutramine treatment (weight loss phase) were randomly assigned to sibutramine or placebo for 18 months (weight maintenance phase). At 24 months, the mean weight loss from baseline was –10.2 ± 9.3 and –4.7 ± 7.2 kg, respectively. Weight loss was accompanied by substantial decreases in the concentrations of serum triglycerides (–25% and –6%, respectively, in the sibutramine and placebo group), insulin (–22% and –3%) and C-peptide (–26% and –12%). Similar results were reported by Van Gaal *et al.* (1998) from an ongoing randomized study.

Two large randomized placebo-controlled trials of treatment with orlistat (an intestinal lipase inhibitor) in subjects on a slightly hypocaloric diet showed some improvement in body weight reduction (about 10% vs 6% in the placebo group), glucose and lipid levels and blood lipids (Sjöström *et al.*, 1998; Davidson *et al.*, 1999).

Dietary modification in female volunteers aiming at reducing estrogen levels. Several studies showed statistically significant reductions of total serum estradiol levels in (mostly) non-obese pre- (Williams *et al.*, 1989) or postmenopausal women (Rose *et al.*, 1987, 1993; Boyar *et al.*, 1988; Prentice *et al.*, 1990) who lost weight (3–4 kg) by following a low-fat diet. Similar results were reported with a low-fat high-fibre diet among pre- (Bagga *et al.*, 1995) and postmenopausal women (Heber *et al.*, 1991). A further study reported a significant reduction in body weight, increased SHBG and decreased total testosterone after a comprehensive dietary modification aiming to increase insulin sensitivity and consumption of phytoestrogens (Berrino *et al.*, 2001). [The Working Group noted that these studies could not distinguish between a possible effect of weight reduction and an independent effect of dietary modification]. Only the three largest studies are summarized here.

Rose *et al.* (1993) randomized 93 postmenopausal breast cancer patients (63 under adjuvant treatment: 50 with tamoxifen alone, 6 chemotherapy alone and 7 with both treatments) to either a dietary intervention group, for which the goal was to reduce total fat intake to 15–20% of total energy, or a control group. The intervention group actually reduced fat intake to about 21% of energy intake and maintained the reduction for 18 months. Control patients showed a sustained increase in body weight (over 1 kg in 18 months), while patients assigned to the low-fat diet showed a reduction in body weight of about 2 kg, which was maintained over the 18-month dietary intervention. Among the 30 patients who did not receive any adjuvant therapy, the 12 in the dietary intervention group exhibited average weight losses of 4.1 and 4.3 kg after 12 and 18 months, respectively, while the 18 patients in the control group did not change their body weight. In the latter group, body weight was significantly correlated with serum estrone sulfate levels ($r = 0.49$). Correlations with other hormone levels were not given. Overall, there was no change in serum estradiol levels over the study period. In 21% of the patients, however, estradiol levels were below the detection limit of the assay (5 pg/mL). Restricting the analysis to patients with initial estradiol levels above 10 pg/mL, those allocated to the low-fat diet showed average

reductions of 20% at six months and 15% at 18 months ($p < 0.005$), while control women did not show any significant change. [The Working Group noted that part of this apparent hormonal effect could be due to regression towards the mean.] There was no significant difference between the serum estrone and estrone sulfate concentrations of the low-fat and control groups. Serum SHBG levels were, as expected, increased by tamoxifen, but were reduced significantly after 12 months (–17%, $p < 0.01$) and 18 months (–23%, $p < 0.05$) on the low-fat diet in patients not receiving tamoxifen (despite a significant weight reduction).

Within the framework of the Women's Health Trial, Prentice *et al.* (1990) studied 73 postmenopausal healthy women (median age 60 years) not using exogenous estrogens, who agreed to reduce dietary fat from customary levels of about 40% energy to a target level of 20%. The duration of dietary intervention was at least 10 weeks (median 14 wk, max. 22 wk). Estimated fat intake decreased from 68.5 g/day to 29.5 g/day (from 37.3% to 20% of their average daily energy intake). Total cholesterol decreased from 234 to 222 mg/dL and the average weight reduction was 3.4 kg, from a mean weight of 69.6 before the intervention to 66.2 kg ($p < 0.001$). Serum concentrations of estrone and estrone sulfate did not change significantly following intervention. On the other hand, total estradiol concentrations were significantly reduced (from 0.71 to 0.63 pg/mL on average, $p < 0.001$), as were the concentrations of bioavailable (unbound and albumin-bound) estradiol (from 0.46 to 0.39 pg/mL, $p = 0.01$) and, to a lesser extent, of SHBG-bound estradiol (from 0.28 to 0.18 pg/mL, $p = 0.03$). Women with high pre-intervention estradiol levels experienced relatively greater reduction, but the authors noted that measurement error in the pre-intervention determinations and consequent regression to the mean in the post-intervention determinations may partially explain this observation. Notwithstanding a significant weight reduction, the serum concentration of SHBG was also reduced after dietary intervention, but not significantly (from 0.07 to 0.02 mg/dL, $p = 0.14$).

It has been hypothesized that the lack of increase in SHBG levels associated with low-fat diets may be attributable to simultaneous increased intake of high glycaemic index carbohydrates, which would prevent the improvement in insulin sensitivity that would be expected following weight reduction (Berrino *et al.*, 2001).

Bagga *et al.* (1995) studied 12 healthy premenopausal women over two months on a very low-fat (10% of energy) and high-fibre (25–35 g/day, provided by whole grain cereals and legumes) *ad libitum* diet. There was a small but statistically significant overall weight loss (–2.0 ± 1.4 kg), a significant reduction in serum levels of estrone (–19 and –18% in the follicular and luteal phase, respectively) and estradiol (–25 and –22%), and no significant change in serum estrone sulfate, SHBG and progesterone. [The Working Group noted that most trials of dietary fibre supplementation show a reduction in estrogen levels, probably due to reduced enterohepatic circulation, suggesting that the effect observed in this study may be independent of weight loss.]

Berrino *et al.* (2001) randomized 104 healthy and mostly non-obese postmenopausal women whose serum testosterone was above the upper tertile of the distribution (> 0.38 ng/mL) to either a dietary intervention or a control group (DIANA study). The diet was available *ad libitum* and designed to reduce plasma insulin levels by lowering animal fat and refined carbohydrates and increasing low-glycaemic index foods and monounsaturated and *n*–3 polyunsaturated fatty acids; phytoestrogen-rich foods, such as soy foods and various seeds, were also increased. The intervention included intensive dietary counselling and specially prepared group meals twice a week over 4.5 months. Control women were not informed of the dietary goals of the study but received a standard recommendation to increase fruit and vegetable consumption. Body weight decreased significantly (4.06 kg vs 0.54 in the control group, $p < 0.0001$). Mean BMI in the intervention group decreased from 26.9 to 25.3 kg/m^2. Waist circumference and hip circumference also decreased significantly (–3.9 and –2.5 cm, respectively, $p < 0.0001$) together with WHR (from 0.82 to 0.80, $p = 0.0045$). SHBG increased from 36.0 to 45.1 nmol/L (25% vs 4% in the control group, $p < 0.0001$) and serum testosterone decreased from 0.41 to 0.33 ng/mL (–20% vs –7% in the control group, $p = 0.0038$). Serum estradiol decreased (–18% vs –6%) but not significantly. Fasting glucose (–5.7% vs –1.2%, $p = 0.026$) and insulin area after an oral glucose tolerance test (–7.7% vs +9.4%, $p = 0.04$) also changed significantly. After adjustment for change in body weight, the differences in changes in hormonal levels between the intervention and control groups were no longer statistically significant (only the difference in fasting glycaemia remained of borderline significance), suggesting that the hormonal effects of dietary intervention were largely mediated through changes in body weight. The increase in consumption of fibre and phytoestrogens, however, may have contributed to the overall effect. Other studies that achieved a similar weight reduction in normal-weight women, in fact, did not observe any effect on SHBG (Rose *et al.*, 1993; Prentice *et al.*, 1990).

Physical activity

Sex hormones in premenopausal women. A few intervention studies have tested effects of chronic exercise on menstrual factors or sex hormones in premenopausal women (Table 22). Sample sizes ranged from 6 to 28 in six of the studies and one study randomized

132 subjects but only 57 completed the study. Thus, the information available from carefully controlled studies is scarce. Most reports on sex hormones in premenopausal women or of an association between physical activity and menstrual disorders have been case reports or observational studies. Most of the latter have been cross-sectional studies of convenience samples, although there have been a few population-based cohort studies.

In a randomized clinical trial of control of energy intake for weight-loss vs weight maintenance (Bullen *et al.*, 1985; Beitins *et al.*, 1991), 28 untrained college-aged women all received the same exercise prescription of 10 miles/-day running plus 3.5 hours/day of sports. All activities took place during eight weeks at a college training camp. Food intake was carefully prescribed and controlled by camp dieticians. Overnight urine was collected daily. During the intervention, eighteen women developed loss of LH surge, 13 experienced delayed menses and 18 developed luteal phase defects. Among women in the weight-loss arm, 75% had delayed menses vs 8% of women in the weight-maintenance arm ($p < 0.005$). Similarly, 81% of women in the weight-loss arm experienced loss of LH surge vs 42% of women in the weight-maintenance group ($p < 0.05$). There was no difference in the incidence of luteal phase defects between the two groups. Six months after termination of the intervention, all subjects were experiencing normal menstrual cycles. The authors interpreted their study as suggesting that vigorous exercise, especially in conjunction with weight loss, can result in reversibly disturbed reproductive function.

In the largest published clinical trial, Bonen (1992) randomized 132 women aged 18–40 years to one of six jogging exercise programmes for either two or four months: < 10 miles/week; 10–20 miles/week; or 20–30 miles/week. There was no control group. Women were followed for nine menstrual cycles. Results were presented only for the 57 who completed the entire study. There was no change in LH concentration, menstrual cycle length or luteal phase length, nor any trend towards increased change in FSH or progesterone with increasing physical activity. There was no change in body weight or percentage body fat, despite a significant increase in maximal aerobic capacity (VO_2max) in all study arms ($p < 0.05$). The authors concluded that recreational running of up to 30 miles/week for four menstrual cycles had no deleterious effect on menstrual cycle. [This study had the benefit of a randomized controlled design with long follow-up, but the considerable drop-out rate (75 out of 132) limits the interpretation of the results.]

In a later study by Williams *et al.* (1999), 15 women were randomly assigned to one of four groups: sedentary control, active control (jogging or cycling 45 minutes/day, 2–3 days/week), training during the follicular phase of the menstrual cycle, or training during the luteal phase of the cycle. The training consisted of twice-daily running or cycling for five days/week. The exercise bouts consisted of running 3.2 km per exercise bout, increasing by 1.2 km per bout each week. The training lasted through two menstrual cycles. Diet was controlled to maintain weight within ± 2 kg of baseline weight. At the end of the intervention, almost half of the trained women showed evidence of abnormal luteal function, including short luteal defects and low urinary progesterone. None experienced loss of LH surge and none had delayed menses or abnormal bleeding. The changes in luteal function were observed in both training groups, suggesting that exercise at any time in the cycle can affect menstrual function.

In the HERITAGE Family Study (An *et al.*, 2000), parents and their adult sons and daughters completed a 20-week endurance training programme (three days/week gradually increased to 75% VO_2max for 50 minutes) on cycle ergometers. On average, SHBG increased by 3.4 nmol/L in the daughters (mean age 25.5 years), but decreased by an average of 7.9 nmol/L in their premenopausal mothers (mean age 48.6 years). Change in SHBG with training was minimal in both fathers and sons.

Insulin-like growth factor (IGF) and insulin-like growth factor binding protein (IGFBP). Information on exercise effects on the IGF system is limited to that gained from a few small intervention studies. An eight-week aerobic exercise programme (three times per week cycling, 1260 kJ (300 kcal) expended) in previously sedentary older subjects (mean age 66 years) increased IGF-I by 19% ($p < 0.01$) in ten men and by 8% in eight women ($p = 0.10$) (Poehlman *et al.*, 1994). No controls were included in this study. The participants did not lose body fat or weight, because the study was designed to maintain energy balance. In a randomized controlled clinical trial in older individuals (31 men, 21 women, mean age 67 years), six months of endurance training three times per week (30–45 minutes/session, gradually increasing intensity to 80–85% heart rate reserve) resulted in a non-significant increase in mean IGF-I from 122.4 ± 8.0 to 131.1 ± 7.6 μg/L (Vitiello *et al.*, 1997). In comparison, IGF-I decreased non-significantly in stretching control subjects from 129.2 ± 11.3 to 125.2 ± 9.3. This result was in spite of a significant decrease in body weight and fat mass in exercisers (compared with no change in controls). A 16-week progressive resistive training programme produced a non-significant decline in IGF-I, from 200.4 ± 60.4 to 190.8 ± 57.6 mg/L in 13 older men (mean age 60 years) (Nicklas *et al.*, 1995). Among nine controls, there was a non-significant increase in IGF-I levels. The assignment to intervention or control group was made by the investigators, based on a judgement of likely success in the intervention.

Table 22. Intervention studies of the association between exercise and menstrual factors in premenopausal women

Reference	Study design	No. of subjects	Mean age or range	Length of follow-up	End-points	Results	Energy balance
Bullen *et al.*, 1984	Cycle ergometry 2 d/wk + running 4 d/wk up to 45 min per session	7	25	2 months	LH, FSH, E2, E3, P, PRL	Decreased urine E3 (4); decreased urine luteal P (2)	
Bullen *et al.*, 1985	Randomized trial weight-loss vs weight-mantenance training – all ran 10 miles/ day + 3.5 h/day sports	28	22	2 menstrual cycles, 6 months follow-up	Menstrual cycles, urine LH, FSH, E3, P	Loss of LH surge (18); delayed menses (13); luteal phase defects (18)	75% of weight-loss vs 8% of weight-maintained groups had delayed menses.
Bonen, 1992	Randomized trial of six exercise programmes, no control group	57	20–36	9 menstrual cycles	LH, FSH, P, menstrual cycle characteristics	No effect	No change in body weight or % body fat
DeCrée *et al.*, 1997a	Progressive training programme, no controls	6	19	3 menstrual cycles	4-OHE, 4-MeOHE, E2, P, LH, PRL	4-MeOHE/4-OHE ratio increased with progressive training ($p < 0.01$)	
DeCrée *et al.*, 1997b	Five-day exercise intervention, no controls	15	18.7	2 menstrual cycles	RBC-COMT activity: 2-MeOHE and 4-MeOHE	Increased COMT activity after intense training	
Loucks *et al.*, 1998	Randomized cross-over trial, energy balance vs restriction, all exercised at intense level	9	21	2 menstrual cycles	LH pulsatility and amplitude	No effect of exercise alone	Low-energy group had 10% ($p < 0.01$) decrease in LH pulse frequency and 36% ($p = 0.03$) increase in LH pulse amplitude
Williams *et al.*, 1999	Randomized controlled trial (active control, passive control, follicular exercise luteal exercise)	15	20	3 menstrual cycles	Luteal abnormalities; urine, LH, P, FSH, E3	Luteal phase defects (4 of 9 exercisers vs 0 of 6 controls); decrease in urinary LH and P	Changes in energy intake may have explained luteal defects

E1, estrone; E2, estradiol; E3, estriol; COMT, catechol-*O*-methyltransferase; 4-OHE, 4-hydroxyestrogen; 2-MeOHE, 2-methoxyestrogen; 4-MeOHE, 4-methoxyestrogen; P, progesterone; PRL, prolactin; FSH, follicle-stimulating hormone; LH, luteinizing hormone

Two weeks of intense endurance training in 10 healthy, young, non-obese men caused a significant increase in IGF-I and GHBP plasma levels with respect to 10 control subjects matched for sex, age and prestudy activity (mainly recreational) (Roelen *et al.*, 1997). In a study of 28 athletes belonging to three swimming teams, four months of increasing training volume (distance swum increased from 5500 to 10 300 m or from 4500 to 6900 m) caused a two-fold increase in IGF-I and IGFBP-3 levels. Free IGF was also increased, but the ratio of IGF-I to IGFBP-3 did not change. IGFBP-1 did not change (Koziris *et al.*, 1999).

Diet in cancer patients. There have been few exercise or weight-loss intervention studies in cancer patients. Studies targeting weight loss in breast cancer patients have had limited evaluation and mixed results.

De Waard *et al.* (1993) randomized 102 postmenopausal women (BMI > 27 kg/m^2) with a recent breast cancer diagnosis to a weight-loss programme involving stepwise reduction in energy intake versus a control group. After one year, median weight loss was 6.0 kg with the intervention ($p < 0.001$).

Using a non-intensive approach, the Mayo Clinic conducted a randomized trial of monthly dietician counselling versus control beginning in the period immediately following breast cancer diagnosis in 107 patients. Median weight increase six months after the start of chemotherapy was 2 kg in the dietary-counselling group versus 3.5 kg in the control group, a non-significant difference (Loprinzi *et al.*, 1996).

Goodwin *et al.* (1998) evaluated a multidisciplinary weight management approach incorporating group dietary sessions, psychological support groups and nutrition and exercise programmes in a non-controlled 61 patient intervention. In breast cancer patients with BMI ≥ 25 kg/m^2, weight loss was 1.63 ± 4.11 kg, and increased aerobic exercise was strongly correlated with successful weight loss.

In a small pilot study, an eight-week exercise–low-fat diet intervention resulted in significant reductions in weight, waist circumference, hip circumference and percentage body fat in nine breast cancer patients aged 40–74 years (McTiernan *et al.*, 1998). Slight, non-significant decreases were observed in serum concentrations of total and free estradiol, estrone sulfate, total testosterone, androstenedione, dehydroepiandrosterone and SHBG.

No multicentre studies aiming at weight reduction with or without exercise as an integral component have been successfully conducted among women with resected breast cancer (Pinto & Maruyama, 1999).

Experimental models

The physiological and hormonal changes that occur with lowering energy intake and/or increasing physical activity affect virtually all organ systems. Qualitatively, the changes seem to readily extrapolate across genotypes and species, presumably because the systems effected are so basic to survival. However, the quantitative extent of the responses may differ with species.

Methodological issues

Controls

One problem in comparing different rodent experiments is the variability observed among animals used as controls in different experiments (Turturro *et al.*, 1995, 1996, 1998). For instance, in the United States National Toxicology Program, chronic bioassay experiments with tight environmental controls are routinely performed so that studies can be compared, but spontaneous liver tumour incidences in the male B6C3F1 mouse have been observed to vary from 20 to 90% (see for example National Toxicology Program, 1997). The control animals are almost always fed *ad libitum*, and similar changes in intake or activity can have very different consequences depending upon the control body weight, complicating interpretation (Turturro *et al.*, 1993). For example, an experimental procedure that results in a 10% lower body weight, either due to exercise or a decrease in food intake, can have adverse effects in control mice with low initial body weight, but when applied to a mouse with a heavy initial body weight can have minimal consequences. This may result from the non-linear relationship between body weight and parameters relevant to the whole animal, such as survival or spontaneous tumour incidence (Turturro *et al.*, 1996; 1998), or specific organs and cells, such as liver cell proliferation and apoptosis (Turturro *et al.*, 2000).

Diets

Research on the physiological and metabolic consequences of underfeeding animals has primarily used dietary restriction protocols where lower levels of all dietary constituents are fed. While this approach does not provide information on the dietary components responsible for a change, dietary restriction studies using laboratory diets do not result in overt malnutrition since standard diets are generous in vitamins and minerals. Malnutrition, such as zinc malnutrition, can be devastating for normal animal physiological function (Good *et al.*, 1982).

Evidence for suboptimal intake of nutrients in some dietary restriction studies has come from studies by Birt *et al.* (1991). In this experiment, dietary restriction was directly compared to energy restriction (the selective reduction of energy-providing components of the diet) in the inhibition of skin papilloma induction (7,12-dimethylbenz[*a*]anthracene-initiated/*O*-tetradecanoylphorbol 13-acetate-promoted) in SENCAR mice. While dietary restriction and energy restriction similarly reduced the incidence and multiplicity of skin tumours, energy restriction resulted in extremely

small lesions, while with dietary restriction the lesions became as large as those observed in control mice. Similarly, in a comprehensive analysis of the studies conducted in another laboratory, it was found using purified diets and selectively reducing single components that only restriction of total energy and not any specific macronutrient (i.e., protein, fat or carbohydrate) or protein source was effective in suppressing spontaneous neoplasms (Shimokawa *et al.*, 1996). These experiments were conducted using semi-purified diets, since similar studies are not possible with cereal-based diets.

Finally, although decreases in dietary intake in dietary restriction studies can be greater than the decreases usually used to control body weight, dietary restriction studies have used such a wide range of models and approaches that they can give only general insights. When short-term (20 days) and chronic (15 months) dietary restriction (of 40%) were compared, many of the consequences for metabolism (e.g., plasma glucose level, plasma insulin, etc.) were qualitatively similar (Dean *et al.*, 1998) if the animal at the beginning of the experiment was not obese. The effects that occur with dietary restriction appear to represent a gradient of responses related to the extent of dietary intake (Turturro *et al.*, 2000).

Decreasing dietary intake for weight control

Many studies on physiological, hormonal and metabolic effects of underfeeding have used dietary restriction. When all components are reduced, it cannot be determined which one is responsible for any particular observation in a model. It is known that significant changes in dietary composition can alter physiological and hormonal parameters (e.g., Iwasaki *et al.*, 1988), but in the present review, the focus is on using diets that do not result in malnutrition.

Size

In rodents, the most prominent effect of lowering dietary or energy intake is decreased body weight gain during growth, resulting in lower body weight and reduced adiposity (i.e., percentage body fat) or fat mass. Using a dietary restriction of 40% (with vitamin supplementation) with cereal-based diets, fat is lost at a higher rate than lean body mass (Duffy *et al.*, 1989, 1990a,b), with emergence of a stable lower body weight after a few weeks, unless the animal was very obese at the start of the study. Rodents grow throughout much of their lifespan, so diet-restricted animals tend to be smaller as well as less fat than those fed *ad libitum*.

Lifespan

One of the most consistent effects of dietary restriction is increased lifespan. For example, using a dietary restriction (with supplementation) of 40%, lifespan, measured as either mean or maximal (average lifespan of the 90th percentile survivors), was extended by 15–30% in four different genotypes of mice and three of rat (Turturro *et al.*, 1999).

Water consumption, body temperature and activity

In animals of average body weight with a dietary restriction (with supplementation) of 40%, daily water consumption is at least twice that of animals fed *ad libitum* (Duffy *et al.*, 1989, 1990a,b; Mittal *et al.*, 2000). Urinary output increases 2–4-fold (depending on age) in male mice using a similar protocol (Taylor *et al.*, 1995). Average body temperature is decreased by approximately 1.5°C in mice and 0.8 °C in rats and the daily range is wider than in animals fed *ad libitum*. Thermotolerance to hot temperatures (measured by ability to survive heat stress) is improved with dietary restriction (Hall *et al.*, 2000a), while ability to withstand cold is decreased. Activity is increased around feeding time, but decreased for the rest of the day in diet-restricted animals.

Cardiac parameters

Blood pressure decreases with dietary restriction (e.g., Haddad *et al.*, 1993, using a 50% dietary restriction), especially in old rats subjected to lifelong dietary restriction of 40% (with supplementation) (Thomas *et al.*, 1993). Short-term 50% dietary restriction or long-term dietary restriction (40%, with supplementation) (Thomas *et al.*, 1993) leads to bradycardia. Short-term intense (50%) dietary restriction decreases the rate of cardiac contraction and relaxation (Hilderman *et al.*, 1996). These decreases are consistent with a smaller heart resulting from dietary restriction, which has been reported with long-term 30% dietary restriction (Oscai & Holloszy, 1970).

Puberty

Puberty can be delayed if intense dietary restriction is started early. For example, in rats maintained at 80–90 g body weight by dietary restriction, puberty was delayed for at least 13 days (Messer & l'Anson, 2000). Refeeding of different dietary intakes allowed puberty to proceed in a dose-dependent manner (time to puberty being inversely related to amount of food consumed). It has been suggested that delay of puberty involves insulin receptor substrate 2 (IRS-2) (a part of the insulin signalling cascade system) in female animals (Burks *et al.*, 2000).

Overall metabolic effects

Overall metabolic parameters, such as oxygen consumption, follow the pattern of physical activity, rising when activity is elevated and falling when it is decreased. Overall metabolism, per gram lean body mass, is approximately the same with dietary restriction and *ad libitum* feeding in mice and rats (Duffy *et al.*, 1989, 1990a,b). Diurnal rhythms of metabolic and most physiological parameters appear to be coordinated to feeding time rather than time of day, while activity is related to light rhythm (Duffy *et al.*, 1995).

Intermediary metabolism

With 40% dietary restriction (with supplementation), the activity of key regulatory enzymes associated with glycolysis decreases, whereas the activities of those associated with gluconeogenesis and with the disposal of ammonia by-products increase. For instance, key glycolytic enzymes, such as pyruvate kinase and alcohol dehydrogenase, decrease in terms of both mRNA expression (Dhahbi *et al.*, 1999) and activity (Feuers *et al.*, 1989; Dhahbi *et al.*, 1999). Increases are also seen in both mRNA expression (Dhahbi *et al.*, 1999) and enzyme activity (Feuers *et al.*, 1989; Dhahbi *et al.*, 1999) of gluconeogenic enzymes, such as phosphoenolpyruvate carboxykinase and liver glucose-6-phosphatase and in insulin-mediated muscle glycogen synthesis activity (Banerjee *et al.*, 1997). mRNA expression of genes important for ammonia detoxification, such as hepatic glutaminase, carbamyl phosphate synthase I and tyrosine transferase, also increases (Dhahbi *et al.*, 1999).

Certain enzymes associated with lipid metabolism, such as malic enzyme and glycerokinase, decrease with 40% dietary restriction (with supplementation), as does the formation of fatty acid epoxides (Allaben *et al.*, 1990). However, there is enhancement of the lipoprotein lipase response in adipocytes to a meal with dietary restriction as well as increased utilization of circulating fatty acids (Sugden *et al.*, 1999).

Although the effects of 40% dietary restriction (with supplementation) on blood glucose and cholesterol levels appear to be modest at best, the effects of dietary restriction on insulin levels are more consistent. Levels of circulating insulin are lowered with dietary restriction (e.g., Feuers *et al.*, 1995), while insulin clearance is increased (Wetter *et al.*, 1999) and the phosphorylation of insulin receptor substrate-1 (IRS-1) in muscle is increased (Dean & Cartee, 2000). Insulin sensitivity increases with dietary restriction in rodents (e.g., Koohestani *et al.*, 1998).

These changes, especially those in the effectiveness of IRS-1, suggest that energy metabolism operates more efficiently in diet-restricted animals compared with those fed *ad libitum*, effectively producing substrates to be utilized in the near term.

Endocrine effects

Studies of the effects of dietary restriction and energy restriction on endocrine status are complicated by the diurnal rhythms seen in hormones, the pulsatile nature of some endocrine phenomena and the sensitivity of hormonal levels to environmental stimuli. This is particularly a problem with studies involving restriction of diet, since the animal may associate the investigator with meals. The animal may then respond with the burst of activity seen at feeding time (Duffy *et al.*, 1989).

A 40% dietary restriction can have such severe effects on the pituitary gland that this treatment has been studied as a model of hypophysectomy (Everitt *et al.*, 1980). One of the most affected pituitary functions involves the lactotrope (Engelman *et al.*, 1993). This also results in inhibition of mammary gland growth and tumorigenesis (Engelman *et al.*, 1994). Growth hormone was 95% inhibited by an energy restriction of 40% after one month, resulting in lowered weight gain; IGF-I was lowered by 12% (Oster *et al.*, 1995). However, with long-term dietary restriction, the ability to produce growth hormone is retained in old animals (Shimokawa *et al.*, 1997).

Leptin, produced by adipocytes, is also reduced by dietary restriction (40%). The lowered level may activate the pituitary–adrenal axis and suppress the gonadal somatotropic and thyroid axes (Aubert *et al.*, 1998; Shimokawa & Higami, 1999). Use of a 40% dietary restriction (with supplementation) of a cereal-based diet also decreased sex-specific steroids and many of the sex differences these steroids support. For example, female- and male-specific cytochrome P450s are inhibited in rats (Leakey *et al.*, 1995). Energy restriction of up to 60% for a month did not appear to affect parathyroid hormone (Ndiaye *et al.*, 1995), except to inhibit its age-related increase. A 40% dietary restriction did not affect calcitonin (Salih *et al.*, 1993). However, a long-term 40% dietary restriction (with supplementation) results in decreased cortical bone mass and mineralization (Banu *et al.*, 1999).

Glucocorticoid levels appear, on the whole, to increase with dietary restriction (Leakey *et al.*, 1998); the major reason for this increase may be the glucogenic role of this hormone rather than a stress response. Plasma glucose and insulin levels fall with a 40% dietary restriction, and free fatty acid metabolism supplies much of the energy for muscle, as noted above. Glucocorticoids, by stimulating apoptosis, provide lipid for this metabolism. This is consistent with the 2–3-h delay in raising plasma glucose levels seen when increasing glucocorticoids (e.g., Shamoon *et al.*, 1981).

Physical activity

Like the effects of restriction of dietary intake, those of physical activity depend upon the system used, as well as the body weight of the animal at the start of the study. For example, in the same rat strain and laboratory, when the control animals weighed approximately 400 g (Holloszy *et al.*, 1985), voluntary wheel-running diminished the beneficial long-term effects on survival expected from the decreased body weight due to this activity. However, when the initial control animal weighed 325 g, the effect on survival of the lower body weight appeared to be overwhelming (Holloszy, 1997). Studies have shown some positive effect of voluntary exercise on survival if the activity began early in life (1–4 months of age). Voluntary exercise started later seems to have little effect on survival or can even have adverse consequences

(Goodrick, 1974). This indicates that timing and level of exercise may be important, as also seen with dietary restriction.

In terms of studying physical activity, because rodents are kept in cages, almost all research has been conducted on relatively inactive animals, particularly for rats. Thus, the following discussion focuses on increases in physical activity rather than inactivity.

Size

One common, though not universal, consequence of exercise is loss of body weight and adiposity (e.g., Garthwaite *et al.*, 1986, using voluntary wheel-running). Fat is lost and there can be an increase in lean body mass that is assumed to be mostly muscle. There seem to be lower levels of basal lipolysis in adipocytes from trained animals, which increase to a greater extent when catecholamines are present (Toode *et al.*, 1993).

Lifespan

The effects of physical activity on rodent lifespan are unclear; there appears to be an increase in mean lifespan but little or no effect in extending maximal lifespan (Poehlman *et al.*, 2001).

Water consumption and body temperature

Since exercise often causes animals to have lower body weight, it will have some of the same effects as dietary restriction or energy restriction. Other than this body weight effect and the immediate need to increase water intake to prevent dehydration, it is not known how exercise affects water consumption, excretion or clearance. Similarly, except for body weight-related effects and the immediate consequences of exercise, little is known of the impact of exercise on body temperature or thermotolerance.

Cardiac parameters

Exercise training (by treadmill for 22 weeks) reduced the tendency for blood pressure to rise in obese rats and reduced blood pressure in normotensive rats (Arvola *et al.*, 1999). This effect appeared to be mediated through vasodilation. There appears to be an increase in heart weight with long-term voluntary exercise (Oscai & Holloszy, 1970), which might be a reaction to increased blood flow during the exercise. Training (treadmill exercise) is reported to maintain cardiac size as animals lose weight (Dowell *et al.*, 1976).

Puberty

Puberty can be suppressed by forced exercise. Comparing voluntary exercise, which does not suppress puberty, and forced wheel-running, only the forced exercise suppressed LH production and interfered with the release of gonadotropin-releasing hormone (Manning & Bronson, 1991).

Overall metabolic effects

There are few reports of resting metabolic rate, which was unaffected by exercise training *per se* in female rats in a study which evaluated the effect over a 23-h period (Ballor, 1991). This approach eliminates the effects of diurnal variation on measurements, which can confound results.

Intermediary metabolism

Chronic exercise appears to have a major effect on glycogenolysis, with increasing fat oxidation as the intensity is increased. In animals trained by either treadmill or a swimming tank, glycogenolysis is inhibited and glycogen levels increase in cardiac and skeletal muscle (Bhagavathi & Devi, 1993). Endurance training increases translocation into muscle of glucose by the glucose transporter protein GLUT-4 and increases gluconeogenesis (Cartee, 1994). These effects may be stimulated by exercise-induced (one hour of swimming) brady-kinin, which may enhance the effect of insulin (Taguchi *et al.*, 2000). Blood glucose levels fall, with blood glucose replenished from fat stores and from proteins (if the exercise is prolonged). Muscle has a large capacity to produce ammonia (from protein and amino acid metabolism), alanine and glutamine (Felig *et al.*, 1970). Levels of intramuscular glutamate drop during exercise and stay low even through prolonged exercise, despite increased glutamate uptake (Graham & MacLean, 1998). Additionally, chronic training seems to result in higher glutamate levels, both when resting and with exercise.

The main function of metabolism appears to be getting substrate to the muscle, for tension development (Stanley & Connett, 1991), without dropping blood glucose so low that there is a problem with brain function.

Endocrine effects

The major endocrine effects of exercise appear to be on growth hormone, catecholamines and the sex steroids.

Plasma levels of growth hormone can increase 100-fold acutely with exercise (especially heavy resistance) in humans (Kraemer *et al.*, 1993) and to a lesser extent in other species such as horse (Golland *et al.*, 1999), sheep (Bell *et al.*, 1983) and trout (Kakizawa *et al.*, 1995). In rodents, this hormone and its metabolites can be inhibited with both acute and chronic involuntary exercise (e.g., Butkus *et al.*, 1995). It is not known what factors are most important in these differences. One speculation is that rodents, whose physical activity is entrained by light, have been fasting since the previous night, and thus are more likely to be affected by the lower levels of nutrients in plasma than other species that have usually eaten a few hours before exercising. An example of the importance of the relationship between feeding and physiological response is that time-of-day of feeding has a significant effect on bone growth in rats (Okano *et al.*, 1999).

Forced swimming exercise elevates levels of catecholamines and

glucocorticoids acutely (e.g., Axelson, 1987), but may also lower the level of response to further activity (so that the activity is not as stressful in trained animals).

Testosterone level is reduced by acute exhausting exercise, but chronic training had no effect on serum and testicular concentrations and seems to only affect the capacity of a testicular interstitial cell suspension to produce testosterone (Harkonen *et al.*, 1990). Moderate forced exercise reduced ovarian steroids and disrupted vaginal cycles in rats (Axelson, 1987). An interesting metabolic effect of estrogen is that it increases fatty acid utilization in male and female rats (Hatta *et al.*, 1988) and improves resistance to fatigue.

Combined exercise and decreased energy intake

Many studies of exercise are actually evaluating exercise combined with uncontrolled weight loss. Because this factor is often overlooked, the attribution of effects to exercise *per se* is often not definitive. Similarly, there can be changes associated with physical activity in some dietary and energy restriction experiments. Surprisingly, few long-term experiments have combined measurement or control of physical activity with controlled dietary intake in rodents, although there are many such combinations that could be tested.

The combination of even voluntary wheel-running and approximately 30% dietary restriction resulted in increased mortality throughout much of the lifespan of rats (Holloszy & Schechtman, 1991). The cause of death of these animals was not evaluated, but further studies using the same model suggested an increase in cardiomyopathy in exercised animals (Holloszy, 1997).

How do I know if I'm doing enough physical activity to stay healthy ?

If you are not sure you're probably doing activities in the light to moderate range on the chart below. You need to work towards adding up 60 minutes of activities a day in periods of at least 10 minutes each.

Time needed depends on effort

Very light effort	Light effort *60 minutes*	Moderate effort *30–60 minutes*	Vigorous effort *20–30 minutes*	Maximum effort
• Strolling	• Light walking	• Brisk walking	• Aerobics	• Sprinting
• Dusting	• Volleyball	• Biking	• Jogging	• Racing
	• Easy gardening	• Raking leaves	• Hockey	
	• Stretching	• Swimming	• Basketball	
		• Dancing	• Fast swimming	
		• Water aerobics	• Fast dancing	

Range needed to stay healthy

Extracted from Handbook for Physical Activity Guide to Health active Living. Canadian Society for Exercise Physiology