Drugs and insulin resistance: clinical methods of evaluation and new pharmacological approaches to metabolism

RICHARD DONNELLY & ANDREW D. MORRIS

Department of Medicine, Stanford University School of Medicine, California, USA and Department of Medicine and Therapeutics, University of Glasgow, UK.

Introduction

Through its integrated actions on carbohydrate, protein and lipid metabolism insulin plays a pivotal role in maintaining glucose homeostasis. Loss of sensitivity to the hormonal effects of insulin, i.e. insulin resistance, affects mainly liver, muscle and adipose tissues in a variety of physiological and pathological conditions (Table 1) but the underlying biochemical mechanisms have not been clearly established [1]. A reduction in insulin-mediated glucose disposal leads to a compensatory hypersecretion of insulin; fasting and postprandial plasma insulin and triglyceride concentrations increase, usually with a reduction in high-density-lipoprotein (HDL) cholesterol. If the pancreatic response is insufficient or defective, insulin resistance may precipitate glucose intolerance. Thus, in type II diabetes mellitus (non-insulin-dependent diabetes, NIDDM) the combination of reduced insulin action and impaired insulin secretion gives rise to hyperglycaemia [2]. By comparison, many obese, non-diabetic subjects are more insulin resistant but avoid diabetes—at least in the short term—by virtue of an adequate β -cell reserve.

Considerable interest has recently focused on putative pathophysiological roles of insulin resistance in the aetiology of various endocrine and cardiovascular disorders [3–6]. While it is fairly clear that resistance to insulin-mediated glucose transport is of primary importance in the development of NIDDM [2], and some rare hereditary syndromes [1], the pathogenetic significance of weak associations between insulin resistance (short of diabetes) and assorted other conditions (Table 1) remains in doubt. What seems likely, however, is that insulin resistance contributes to some of the clinical, biochemical and metabolic features that often complicate these conditions—for example, glucose intolerance during pregnancy, hypertriglyceridaemia in obesity and diabetes, and hyperglycaemia in patients with the so-called syndrome X [3].

Numerous drugs affect glucose tolerance [7], usually via changes in insulin secretion, while some exert peripheral effects on insulin target tissues. In general, the nature and magnitude of drug effects on insulin-dependent metabolic pathways have been poorly characterised and sometimes misrepresented. Non-pharmacological measures that improve insulin

sensitivity, e.g. weight loss and physical exercise, are moderately effective in lowering glucose and triglyceride levels but few drugs, other than the biguanides, enhance insulin signaling. However, there are now several groups of novel pharmacological agents, including insulin-sensitising drugs, approaching clinical development with potential therapeutic applications in NIDDM, obesity and some forms of dyslipidaemia. Thus, the purpose of this review is to describe some of the clinical methods available for evaluating drugs that act on insulin-dependent metabolic pathways; to clarify the effects of some existing compounds on insulin resistance; and to discuss future prospects for a new generation of metabolic agents.

Clinical measurements of insulin sensitivity

The two major target tissues of insulin action are muscle and liver. Although the insulin-receptor interaction elicits a complex array of biochemical responses, by convention insulin sensitivity refers only to glucose metabolism, i.e. the effects of insulin on hepatic glucose production and hepatic and peripheral glucose utilization (both oxidative and non-oxidative). Elevated basal free fatty acid (FFA) levels and failure of insulin to suppress FFA production reflect insulin resistance in respect of lipid metabolism [8], which is often secondary to impaired glucose oxidation. Thus, pathways of glucose, lipid and aminoacid metabolism are closely inter-linked—resistance to glucose usually implies resistance to lipid and aminoacid metabolism as well [1]—but other effects of insulin, e.g. on salt and water balance, may be spared in classic insulin resistant states. For example, it is still unclear whether insulin resistance extends to potassium disposal.

In the 1930s, Himsworth introduced the first standard approach to measuring insulin sensitivity *in vivo* [9, 10]. Two oral glucose tolerance tests were performed on individual diabetic subjects: one with and a second without an i.v. insulin injection. Insulin sensitivity was expressed as the ratio of the areas under the respective glucose tolerance curves, but without direct measurements of plasma insulin concentration

Table 1 Physiological and pathological conditions associated with insulin resistance and its metabolic sequelae

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<i>Physiological states</i>
Puberty
Old age
Pregnancy
Fasting/starvation
Physical inactivity
<i>Insulin resistance secondary to another endocrine/metabolic disorder</i>
NIDDM
Obesity
Rare genetic disorders of severe insulin resistance, e.g. Type A syndrome, Leprechaunism
Ketoacidosis
Cushing's syndrome
Acromegaly
Pheochromocytoma
Thyrotoxicosis
Hyperinsulinaemia, e.g. insulinoma, exogenous-type I diabetes
Oestrogen/steroid therapy
<i>Insulin resistance associated with other diseases</i>
Stress, e.g. fever, sepsis
Uraemia
Cirrhosis
Hypertension
Cardiac failure
Atherosclerotic vascular disease

Himsworth could only infer differences in tissue insulin sensitivity, e.g. between type I (insulin-dependent) and type II (non-insulin-dependent) diabetics [10]. Thus, he was unable to exclude differential effects of exogenous insulin on glucose absorption and suppression of endogenous insulin release, or differences due to variation in insulin clearance. Direct assessment of insulin sensitivity ideally requires a stable, quantitative relationship between plasma insulin concentration and some measurable insulin-dependent metabolic response. Three techniques, each with a different set of limitations, fulfill this requirement, and have found widespread use in clinical research: the euglycaemic clamp, the insulin suppression test, and the frequently-sampled i.v. glucose tolerance test with model-dependent analysis.

Oral glucose tolerance test (OGTT)

Inappropriately elevated plasma insulin concentrations, either following an overnight fast or 2 h after an oral or i.v. glucose load, in the face of normal or above-normal plasma glucose concentrations, is generally accepted as evidence for decreased overall tissue sensitivity to insulin. The fasting plasma insulin concentration is widely used as a simple index of insulin sensitivity in large epidemiological studies. The OGTT and its associated insulin response can provide a simple, albeit indirect, estimation of whole-body insulin sensitivity. However, the heterogeneity of OGTT profiles in both normal and diabetic subjects has been widely emphasised [11], and it is essentially impossible to draw conclusions about insulin sensitivity when an intervention (e.g. drug) or disease (e.g. NIDDM) under investigation is poten-

tially associated with changes in insulin secretion as well as insulin action. For example, changes in peripheral insulin sensitivity affect plasma glucose concentrations, which in turn affect glucose-induced insulin release, making it impossible to differentiate between pancreatic and extra-pancreatic effects. Thus, an OGTT characterises the net effect of several glucoregulatory mechanisms but provides only limited information about individual pathways and insulin sensitivity; it is nevertheless useful for larger studies, when absolute measurements of insulin-mediated glucose uptake are not required, and when the study design uses individuals as self-controls.

Intravenous glucose tolerance test (IVGTT)

A conventional IVGTT characterises the early and late phases of insulin secretion following bolus injection of a glucose load. The net fractional glucose disappearance rate (K value) is easily calculated from the profile of plasma glucose concentrations, but differences in K cannot distinguish between effects on insulin secretion and insulin action.

Bergman *et al.* have described a modification of the IVGTT—the so-called ‘minimal model’ or frequently-sampled IVGTT—that provides a quantitative index of insulin sensitivity [12]. An i.v. bolus of glucose is followed 20 min later by an injection of insulin or tolbutamide 500 mg; some 30 blood samples are then collected over 180 min for glucose and insulin measurements which are analysed using computer-based model predictions of insulin's effect on glucose kinetics. The crucial piece of software, marketed and apparently understood only by the authors, calculates an index of insulin sensitivity with

few details of how the measurement was derived. Despite the obvious complexity of this method, parameters derived from the minimal model correlate well with other techniques [13].

Euglycaemic hyperinsulinaemic clamp

This widely popular, though labour intensive, method was developed by Andres & DeFronzo [14, 15]. With the aims of over-riding the normal glucose-insulin feedback mechanism and maintaining stable plasma glucose and insulin levels, this technique uses a constant i.v. infusion of soluble insulin (e.g. $1.5 \mu\text{u kg}^{-1} \text{min}^{-1}$) to increase circulating insulin concentrations by about $100 \mu\text{u ml}^{-1}$, and a variable infusion of 20% glucose to maintain normoglycaemia ($5\text{--}6 \text{ mmol l}^{-1}$). Although the increase in plasma insulin concentration is almost immediate, there is a variable time lag of 1–2 h before the maximal hypoglycaemic stimulus is observed. Thus, during a typical clamp, the glucose infusion rate is increased progressively until a steady state equilibrium is achieved, usually at around 120 min (Figure 1). Calculations of insulin sensitivity are based on a 30–40 min period of steady state infusion when the rate of glucose input approximates to whole-body glucose disposal (M value, expressed in $\text{mg glucose kg}^{-1} \text{min}^{-1}$). Thus, it is usually necessary to maintain the infusions for at least 3 h.

The glucose infusion rate can be adjusted manually in response to rapid measurements of plasma glucose at the bedside, or automatically with a computerised glucose-controlled infusion system (Biostator) [16].

Figure 1 Representative euglycaemic hyperinsulinaemic clamp in a healthy volunteer. The exogenous insulin infusion ($1.5 \mu\text{u kg}^{-1} \text{min}^{-1}$) produces a 'steady state' plasma insulin concentration approximately $100 \mu\text{u ml}^{-1}$ above basal fasting value (a). A dose-dependent fall in serum potassium is observed (b). Whole-body glucose disposal, M , in $\text{mg glucose kg}^{-1} \text{min}^{-1}$ is calculated during the last 40 min of 'steady state' at the end of the 3 h clamp. ▼ = plasma glucose concentration (c).

Manual corrections are perfectly adequate but can be influenced by subjective bias (by up to 15%), so in clinical pharmacological studies it is still important that the investigator remains blind to drug administration. Rebound hypoglycaemia is a potential complication if glucose and insulin infusions are stopped simultaneously. This is easily avoided by continuing i.v. glucose for an extra 20 min and feeding the subject at the end of the procedure. *In vivo* insulin infusion with maintenance of euglycaemia causes a dose-dependent fall in plasma potassium concentrations by promoting potassium uptake into the liver and peripheral tissues. However, below a threshold potassium level the splanchnic potassium exchange switches from a net uptake to a net release, so preventing further hypokalaemia [17] (Figure 1). Thus, potassium replacement in the infusate is usually unnecessary.

The clamp provides an overall measurement of whole-body insulin sensitivity, which represents the net balance of peripheral glucose utilization and endogenous hepatic glucose production, but there has to be some concern that measurements derived under such artificial conditions might not be a true reflection of physiological responses to insulin. In insulin resistant states, especially NIDDM, it is unlikely that the insulin stimulus will completely suppress glucose secretion from the liver. By combining the clamp with a constant infusion of labelled tracer (either tritiated or deuterated glucose), it is possible to quantify rates of glucose appearance (R_a) and disappearance (R_d) which correspond to hepatic glucose production and peripheral glucose utilization respectively [18]. These methods depend upon steady-state conditions at the time of blood sampling but some investigators doubt whether a true equilibrium is achieved after 3 h, raising questions about the accuracy of these measurements.

The glucose taken-up by skeletal muscle is processed through oxidative (i.e. glycolytic) or non-oxidative (e.g. glycogen synthetic) pathways of metabolism, and by combining the euglycaemic clamp with a constant infusion of labelled glucose and indirect calorimetry [19], it is possible to quantify further peripheral glucose utilization in terms of oxidative and non-oxidative rates of disposal.

Insulin suppression test (IST)

The principle of the insulin (or pancreatic) suppression test involves pharmacological inhibition of endogenous insulin secretion and other counter-regulatory hormones (e.g. glucagon and growth hormone) during constant infusions of glucose and insulin. Endocrine suppression was originally achieved using i.v. infusions of adrenaline and propranolol [20] but lately this has been replaced by somatostatin [21] or the somatostatin analogue, octreotide, which is more widely available and appears to be better tolerated. As with the clamp, plasma glucose and insulin concentrations gradually reach a plateau (Figure 2); the steady state plasma glucose concentration (SSPG) is a reflection of whole body insulin sensitivity.

Figure 2 Representative insulin suppression test in a healthy subject. After approximately 120 min infusion of insulin, glucose and somatostatin (or octreotide) the steady state plasma insulin (SSPI) and steady state plasma glucose (SSPG) are calculated. $\blacktriangle$ = plasma glucose (mmol l^{-1}), $\circ$ = plasma insulin ($\mu\text{mol l}^{-1}$).

Model-derived parameters of insulin sensitivity

Mathematical models have been developed to describe the complex kinetics of glucose and insulin under physiological conditions, and to derive parameters for peripheral insulin sensitivity and β -cell dysfunction to glucose-induced insulin secretion. The technique of HOMA (homeostatic model assessment), for example, uses measurements of fasting plasma glucose and insulin to derive an index of insulin sensitivity (S) which shows good correlation with parameters obtained from infusion techniques [22, 23]. Since basal (fasting) insulin concentrations are particularly subject to assay variability, Hosker *et al.* [24] have attempted to improve on the assessment of insulin sensitivity from HOMA by stimulating endogenous insulin secretion by a constant infusion of glucose. Thus, CIGMA (continuous infusion of glucose with model assessment) defines insulin sensitivity from the steady state levels of glucose and (endogenous) insulin after 2 h. These 'structural' model predictions have many advantages—not least that measurements reflect physiological responses to endogenous insulin, rather than 'pharmacological' infusions—but as with all models there are some inherent assumptions that may be over-simplistic, e.g. that various tissues in different subjects behave similarly with respect to glucose metabolism, and many of the variables used to calculate insulin sensitivity are not individualised for each subject.

'Arterialized' blood sampling

For both of the principal infusion techniques for assessing insulin sensitivity it has been emphasised that plasma glucose levels should preferably be measured from the arterial side of the circulation. Thus, during hyperinsulinaemic stimulation glucose extraction across the capillary bed is high, resulting in a significant A-V difference in glucose concentration. To avoid arterial puncture, many investigators sample blood from a dorsal hand vein surrounded by a heated box. The hand has relatively little muscle

bulk, and warm-air heating (60°C) creates a physiological A-V shunt, so theoretically minimising glucose extraction. In clinical evaluations of this manoeuvre, the box is clearly effective in 'arterializing' venous blood [25, 26]; for example, in our own hands, glucose levels were $0.8\text{--}1.3\text{ mmol l}^{-1}$ higher than in ordinary venous blood collected from the antecubital fossa, and the corresponding oxygen saturations were 96% vs 78%. This technique may well find wider research applications as a reasonable substitute for direct arterial puncture.

Variability in measurements of insulin sensitivity

Even amongst non-diabetic subjects there is wide inter-subject variability in insulin sensitivity. For example, in healthy, non-obese male volunteers ($n = 36$), insulin sensitivity measured by the euglycaemic clamp ranged from $5.6\text{--}16.2\text{ mg kg}^{-1}\text{ min}^{-1}$ [27, unpublished]; these differences were not readily explainable by variations in body weight, age, physical fitness or fat distribution but the intra-subject variability was less than 9% over 2 weeks. In patient populations the between-subject variability is likely to be much higher, yet many published studies—including drug evaluations—have opted for parallel group comparisons. This is undoubtedly a major weakness; the techniques for assessing differences in insulin sensitivity are most useful when subjects serve as their own controls.

Controversial drug effects on peripheral insulin sensitivity

The mechanism by which many drugs affect glucose tolerance is usually via a change in insulin secretion [7], but there are some notable examples of drugs that have been associated with effects on peripheral insulin sensitivity.

Metformin

Metformin is widely used in the treatment of NIDDM (though it is not yet licensed in the US), both as monotherapy and in combination with sulphonylureas. It is generally agreed that the glucose-lowering effect occurs without stimulation of insulin secretion and several antidiabetic mechanisms have been implicated, including delayed gastrointestinal absorption of glucose [28], enhanced tissue sensitivity to insulin [29], and suppression of hepatic glucose output via inhibition of gluconeogenesis [30]. Although the principal underlying mechanism remains unclear, several studies have shown increases in insulin-mediated glucose uptake and oxidative metabolism in muscle [31–33]. However, this is not a universal finding [34], and many believe that the hypoglycaemic effect is largely due to a reduction in hepatic glucose output [30]. Consistent with its putative insulin-mimetic actions, metformin has other beneficial effects on lowering triglyceride levels and body weight.

Sulphonylureas

These drugs form the mainstay of oral therapy for NIDDM but there is still some debate about whether sulphonylureas lower glucose levels by mechanisms in addition to stimulation of insulin secretion [35]. Part of the controversy about extrapancreatic effects stems from studies evaluating insulin responses during chronic therapy; hypoglycaemic effects were observed with little or no change in plasma insulin concentrations [36]. In addition, several studies have demonstrated improved insulin sensitivity [37, 38] and increased insulin receptor binding [39]. However, at present, the balance of evidence is probably against significant extrapancreatic effects; for example, sulphonylureas do not lower plasma glucose in insulin-dependent diabetics or patients with NIDDM rendered insulinopaenic with somatostatin [40], and it is quite possible that the apparent increase in insulin sensitivity is simply due to improved glycaemic control rather than any direct effect of the drug itself.

Angiotensin converting enzyme (ACE) inhibitors

Incidental observations appeared in the mid-1980s suggesting an improvement in glucose tolerance after treatment with an ACE inhibitor. Anecdotal reports from diabetic clinics also described a reduction in insulin requirements or discontinuation of oral hypoglycaemic therapy after the introduction of either captopril or enalapril [41, 42]; even hypoglycaemic episodes were attributed to ACE inhibition [43].

The most widely quoted study of ACE inhibitors and insulin sensitivity is by Pollare *et al.* [44]. This study was performed in 50 non-diabetic hypertensive subjects to compare captopril and hydrochlorothiazide, each administered for 16 weeks, in a randomised, cross-over design. Despite a 4-week washout phase, carry-over effects hampered the intended analysis, so results were presented for two parallel groups during the first treatment phase only. There was no difference in fasting plasma glucose and insulin levels, or glycosylated haemoglobin, but insulin-stimulated glucose disposal (M) increased marginally from 5.7 to 6.3 mg kg⁻¹ min⁻¹ after captopril, compared with a change from 6.4 to 5.7 with the diuretic. In a similar study with enalapril [45], haemoglobin A_{1c} decreased after 4 weeks of the ACE inhibitor but there was no change in M.

Since skeletal muscle blood flow is an important determinant of glucose extraction rate [46], it is possible that vasodilation *per se* might improve insulin sensitivity. Theoretically this could explain the effects seen with ACE inhibitors but it is then difficult to explain why other vasodilator drugs have no effect on glucose transport. Although angiotensin II has no effect on insulin sensitivity, at least in normal subjects [47], increased kinin levels following ACE inhibition enhance glucose transport [48].

Few, if any, studies of the metabolic actions of ACE inhibitors have been adequately controlled, and there are no direct comparisons with placebo. Of 33 studies in which some parameter of glycaemic control

was assessed in patients with NIDDM and hypertension receiving an ACE inhibitor, improvements were noted in only 11: 3 out of 9 studies with enalapril and 8 of 24 with captopril [49]. Thus, it seems unlikely that ACE inhibitors have any clinically significant effect on glucose metabolism.

Thiazide diuretics

Peripheral insulin resistance has been implicated as one possible mechanism of thiazide-induced glucose intolerance. However, a recent cross-over study using the euglycaemic clamp showed no effect of bendrofluazide 1.25 mg and 5 mg on insulin sensitivity after 12 weeks [50].

New pharmacological approaches to the metabolic defects of NIDDM and obesity

NIDDM is characterised by a triad of metabolic abnormalities associated with hyperglycaemia: peripheral and hepatic insulin resistance, impaired insulin secretion and hepatic glucose overproduction. There is some debate about which disorder arises first but insulin resistance, affecting multiple pathways of glucose and lipid metabolism, is generally regarded as the principal defect [2]. It therefore seems somewhat anomalous that sulphonylurea drugs, which are primarily insulin secretagogues, should form the mainstay of oral therapy; the ideal treatment should logically focus on reversing the peripheral defects in glucose and lipid oxidation. This is perhaps supported by the relatively high rates of secondary failure of sulphonylurea therapy [51], which presumably reflect islet-cell exhaustion in the face of sustained tissue insulin resistance. The last decade has seen enormous progress in defining the biochemistry of NIDDM, and this has led to a variety of pharmacological innovations (Table 2), some of which are now undergoing early clinical evaluation in patients with glucose intolerance, NIDDM and obesity.

Insulin-sensitizing agents

The thiazolidinedione derivatives represent a new structural class of antidiabetic compounds. Included in this group are CS-045, ciglitazone, pioglitazone, and englitazone. At least three are presently undergoing phase I and II studies, and preliminary reports have demonstrated hypoglycaemic efficacy in patients with NIDDM [52–54]. Analogues of this class lower plasma glucose, insulin and triglyceride levels in a variety of rodent models in which insulin resistance is a conspicuous feature, and biochemical studies have shown insulin-mimetic effects in each of the key target tissues [55]. Thus, thiazolidinediones increase glucose oxidation in adipose tissue and muscle, increase glycogen and lipid synthesis from glucose, and decrease glycogenolysis [56, 57]. *In vitro* studies have shown that this group of drugs act as insulin-sensitizers; for example, insulin-stimulated uptake of

Table 2 New pharmacological approaches to manipulating insulin-dependent pathways of glucose and lipid metabolism in NIDDM and obesity

<i>Insulin-sensitising agents</i>	<i>β_3-adrenoceptor agonists</i>	<i>Inhibitors of lipolysis</i>	<i>Inhibitors of fatty acid oxidation</i>
Thiazolidinedione derivatives: CS-045 Ciglitazone pioglitazone englitazone CP 68,722	BRL 37344 BRL 26830A ICI D7114	Nicotinic acid derivatives: Acipimox Adenosine (A1) receptor agonists: GR79236	CPT-1 inhibitors: Colomixir Etomoxir TDGA SDZ 51-641
Dichloroacetate derivatives			
Vanadate analogues			

deoxyglucose in isolated adipocytes from KKA^y mice was markedly more sensitive to insulin after pretreatment of these insulin resistant animals with pioglitazone [55], and thiazolidinedione effects (like those of metformin) are dependent upon the presence of insulin [58]. In addition to their hypoglycaemic effects, these drugs have other properties that may be particularly useful in patients with NIDDM, including effects on HDL-cholesterol and possibly also blood pressure [59].

The insulin-mimetic effects of vanadate, the salt of a trace element found in most tissues, have attracted pharmacological interest [60]. Vanadate is an essential nutrient for normal cell growth and development but its physiological function remains unknown. In recent years, vanadate has been shown to mimic most of the metabolic effects of insulin: for example, in various cell systems and after oral administration to streptozotocin-treated rats, vanadate increased glucose uptake and oxidation in muscle and fat, inhibited lipolysis and stimulated glycogen synthase activity [60–63]. The underlying biochemical mechanisms have not been clearly established but there is evidence that vanadate by-passes some of the critical steps in insulin action and enhances glucose transport and oxidation in insulin-resistant tissues that have become totally refractory to the effects of insulin itself [60]. Thus, vanadate serves as a useful model for drug developments aimed at improving insulin-mediated responses.

β_3 -adrenoceptor agonists

The classification of β -adrenoceptors that mediate catecholamine-induced lipolysis has been the subject of intense debate. Lipolytic effects have traditionally been defined in terms of β_1 - and β_2 -adrenoceptor-mediated activation but there have been an increasing number of reports showing that this simple subdivision may not be entirely correct. In particular, binding studies have identified a group of atypical (β_3 -) adrenoceptors in fat tissue, and the human β_3 -receptor gene has now been cloned and characterised [64].

β -adrenoceptor-mediated metabolic responses in isolated adipocytes—i.e. oxygen and substrate consumption, lipolysis and cAMP accumulation—seem to be predominantly mediated by atypical β -adrenoceptors [65], and clinical studies have provided supportive evidence for a functionally distinct subgroup of β_3 -adrenoceptors which mediate thermogenic responses to isoprenaline [66]. These observations have raised interesting possibilities with regard to the potential therapeutic effects of selective β_3 -adrenoceptor agonist drugs in obesity and NIDDM [67].

In obesity, there are two ways of achieving weight loss: by reducing food intake or by stimulating heat production and energy expenditure. The idea of using thermogenic agents to increase energy consumption is far from new but most such agents, e.g. thyroxine or sympathomimetic drugs, elicit unacceptable cardiovascular side-effects and cause excessive depletion of tissue proteins. Adipose tissue is an important regulator of thermogenesis and it now appears that β_3 -adrenoceptors play a significant role in determining the rate of energy consumption. A number of β_3 -adrenoceptor agonists have undergone preliminary clinical evaluation in insulin resistant states, particularly obesity (Table 2).

BRL 37344 (the active metabolite of BRL 35135) is an example of a potent β_3 -adrenoceptor agonist which causes dose-related increases in energy expenditure and weight loss in rodent models [68]. In lean, healthy humans, oral administration of BRL 35135 produced significant increases in thermogenesis, as assessed by indirect calorimetry [68], and in obese subjects there were additional effects on glucose tolerance and insulin sensitivity [69, 70]. Thus, after 10 days treatment, there was a 25% improvement in glucose tolerance due predominantly to an increase in non-oxidative glucose disposal; however, for a drug that is purported to stimulate energy consumption, it is perhaps surprising that glucose oxidation rates were unaffected [70]. The effects of BRL 35135 on insulin sensitivity have also been assessed in patients with NIDDM. Although 10 days treatment had no effect on body weight, whole-body insulin sensitivity

increased by 90% [68]. Similar profiles have been reported with the related compounds, BRL 26830A [71] and ICI D7114 [72].

Inhibitors of fatty acid oxidation

Elevation of FFA levels is a well recognised feature of NIDDM, and there is evidence that this may be causally related to hyperglycaemia. Fatty acid release from adipocytes is dependent upon the net balance between lipolysis and re-esterification. In NIDDM, there is resistance to insulin-mediated suppression of lipolysis (via hormone-sensitive lipase) and resistance to glucose transport, which is the rate-limiting step in FFA re-esterification. The principal mechanism by which FFAs may be causal in hyperglycaemia is in stimulating gluconeogenesis in the liver. In addition, Randle *et al.* [73] first proposed that increased FFA levels lead to insulin resistance in skeletal muscle. Their hypothesis was based on two observations: that fatty acids decreased the rate of uptake of L-arabinose (a glucose analogue that is transported into cells but not metabolised), and decreased the rate of glucose phosphorylation. The mechanism whereby FFAs decrease glucose transporter sensitivity is not entirely clear but the effect on glucose phosphorylation is due to increased oxidation of FFAs. Thus, increased FFA oxidation inhibits glucose oxidation via inhibition of pyruvate dehydrogenase. This results in a decrease in glucose utilisation [73].

Randle and colleagues also proposed another mechanism whereby FFA oxidation leads to a reduction in glucose uptake. Oxidation of FFAs leads to an increased rate of production of acetyl-CoA, which in turn leads to increased levels of citrate. The increase in citrate then inhibits the enzyme phosphofructokinase, leading to an elevation of glucose-6-phosphate and inhibition of hexokinase. This inhibitory effect on hexokinase may decrease glucose uptake to a greater extent than the inhibition of glucose oxidation [74]. Thus, the hypothesis that a glucose-fatty acid cycle operates suggests that limiting the supply of fatty acids to peripheral tissues should promote carbohydrate utilisation.

Insulin therapy and other inhibitors of lipolysis, e.g. nicotinic acid and its analogue acipimox, reduce levels of FFA and glucose [75]. Clinical trials with acipimox have shown useful effects on triglyceride levels and glycaemia [75, 76], but rebound effects with these antilipolytic agents have focused attention on different approaches to lowering elevated levels of FFAs. One approach is with novel adenosine A-1 receptor agonists, e.g. GR79236 [77], which inhibit catecholamine-induced lipolysis. In addition, a series of inhibitors of fatty acid oxidation are presently in various stages of development (Table 2). In particular, a group of drugs that irreversibly inhibit a key mitochondrial enzyme involved in long-chain fatty acid oxidation, carnitine palmitoyltransferase (CPT) 1, have entered preliminary studies in humans.

The CPT 1 inhibitors include clomoxir, etomoxir and methyl 2-tetradecylglycidate (TDGA) [78]. In humans, TDGA was effective in lowering glucose

levels in both type I and type II diabetics, and reversed ketonuria [79], but clinical trials were suspended due to cardiac hypertrophy in long-term animal toxicology studies. Etomoxir also inhibits fatty acid oxidation, ketogenesis and gluconeogenesis from long-chain FFAs. A 20% reduction in fasting plasma glucose levels was observed in patients with NIDDM, together with a 60% fall in 3-hydroxybutyrate and triglycerides [80]. Euglycaemic clamp studies with etomoxir in patients with NIDDM showed a 33% increase in insulin-mediated glucose disposal [81]. There is additional evidence that etomoxir lowers hepatic glucose production and improves the type IV pattern of dyslipidaemia often associated with diabetes [82].

Thus, present evidence suggests that fatty acid oxidation inhibitors may be particularly effective in lowering hepatic glucose production and enhancing peripheral insulin action in patients with NIDDM, and as antiketogenic agents in insulin-dependent diabetes. Potential adverse effects on heart muscle make liver targeting of fatty acid oxidation a priority for future drug developments, e.g. SDZ 51-641.

Dichloroacetate

Although dichloroacetate (DCA) itself is not new, it represents a potentially novel class of oral hypoglycaemic agents. DCA has no effect on insulin secretion but reduces plasma glucose levels via inhibition of hepatic glucose production and stimulation of peripheral utilization [83]. The peripheral effect is due mainly to stimulation of pyruvate dehydrogenase, which is the rate-limiting enzyme of glucose oxidation. In patients with NIDDM, short-term treatment with DCA was associated with a 24% reduction in glucose, and a significant fall in triglycerides [84]. Clinical trials with DCA were discontinued due to one case of reversible neuropathy, but new DCA derivatives are now becoming available [85].

Conclusions

Peripheral and hepatic insulin resistance is of primary pathophysiological importance in the aetiology of NIDDM, and may contribute to some of the clinical, biochemical and metabolic abnormalities that frequently complicate other disorders. Favourable experience with metformin, as well as the known metabolic effects of weight loss and physical exercise, testify to the potential therapeutic advantages of new drugs that modify insulin-dependent pathways of metabolism, either in the liver or peripheral tissues, or both. A number of agents have begun to enter clinical phases of development, and clinical pharmacologists are likely to become increasingly involved in assessing the *in vivo* pharmacodynamic effects of these drugs on specific biochemical pathways. In addition to improved glucose tolerance, some of the newer agents may have beneficial effects on plasma lipids, body weight and ketogenesis. Several rela-

tively non-invasive techniques are available to evaluate drug effects on insulin-dependent glucose and lipid metabolism. These methods can quantify hepatic glucose production and (when coupled with gaseous exchange measurements) peripheral glucose disposal through oxidative and non-oxidative routes of metabolism. The euglycaemic clamp is highly reproducible but prone to subjective bias; study designs should take account of the wide inter-subject variability in insulin sensitivity and the need for strict blinding of the investigator if glucose administration is controlled manually. Some of the simpler methods, e.g. an OGTT, though useful for assessing the net effect on

glucose tolerance, cannot reliably distinguish between drug-induced changes in insulin secretion and insulin action. Finally, newer techniques that rely on magnetic resonance spectroscopy provide an opportunity to monitor specific biochemical pathways and rate-limiting enzymes in human studies [86–88].

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