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Basics of Clinical Metabolic Research

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Metabolic diseases, particularly obesity, dyslipidaemia and type 2 diabetes mellitus (T2DM), as well as conditions of increased risk for these diseases such as factors of the (dys)metabolic syndrome have become dramatically more prevalent over the last decade. Both in the industrialised world and, even more so, in developing regions and countries – which feature rapidly increasing economies and are adopting the so-called Western lifestyle – these diseases, particularly overweightness and obesity, are a growing health problem (Kopelman 2000). It is assumed that T2DM could be the largest epidemic in human history (Zimmet 2005) as more than 190 million people worldwide are diabetic and more than 300 million suffer from impaired glucose tolerance, the immediate pre-diabetic state. Recent calculations suggest that in the year 2025 more than 300 million people will have overt diabetes, mainly T2DM, with excessive growth in developing countries (King et al. 1998).

Diabetes mellitus is already the leading cause of blindness among working-age adults, of end-stage renal disease and of non-traumatic loss of limb (Ullbrecht et al. 2004; Williamson et al. 2004). The global mortality attributable to diabetes in the year 2000 was estimated to be 2.9 million deaths, which was 5.2 % of all death. Thus diabetes is the fifth leading cause of death globally. About 2–3 % of the population in low-economy countries and up to 8 % in the United States and Canada die because of diabetes (Roglic et al. 2005). The costs caused by diabetes are enormous. Currently diabetes care accounts for 2–7 % of the total national health care budgets in Western countries, amounting to \$132 billion in the United States in 2002 (ADA 2003). These data underline the importance of understanding the cellular mechanisms of the causes and complications of type 2 diabetes mellitus in order to offer better targeted and more effective treatment or even prevention of the disease.

Over the last decades, we have learned a lot from *in vitro* experiments in isolated tissues and cell cultures, but especially from *in vivo* studies in mouse models of modified insulin secretion or action (Nandi et al. 2004). Nevertheless, the phenotypes resulting from the various tissue-specific knockout or overexpression mouse models of diabetes or metabolic diseases do not generally resemble the phenotypes of corresponding diseases in humans. In addition to species differences and technical limitations of metabolic studies in small animals,

this observation can likely be explained by gene-environment interactions influencing the human phenotypes of metabolic disorders. This makes detailed studies in humans under *in vivo* conditions mandatory.

Starting from simple endocrine and metabolic stimulation or suppression tests such as the insulin and glucagon administration or glucose tolerance tests, a series of more sophisticated tests has been developed over the last three decades. This development is mirrored by the near-exponential rise in original papers and reviews on the topics ‘diabetes’ and ‘metabolic diseases’ in peer-reviewed journals since 1970 (Figure 1.1). Among those publications, some key methodological papers speeded up the development of clinical metabolic research. These key papers include the description and validation of standardised insulin sensitivity and secretion tests such as the glucose clamp (Tobin et al. 1979) and minimal modeling of glucose and insulin concentrations during the intravenous glucose tolerance test (Bergman et al. 1981). Later on, tissue-specific metabolism became accessible by applying *in vivo* multi-nuclear magnetic resonance spectroscopy (MRS) (Shulman et al. 1990; Krssak et al. 1999) or positron emission tomography (PET) (Nuutila et al. 1993). Novel applications of stable isotopes as labels of molecules, e.g. $^2\text{H}_2\text{O}$, later allowed quantification of complex metabolic fluxes such as rates of gluconeogenesis from different sources and glycogenolysis from one single blood sample (Landau et al. 1995) (NOTE: I would like to express my grief for my long-term mentor and friend, Professor Landau, an outstanding researcher and scientist in the field of metabolism, who passed away during the printing of this book). Combining the different techniques, e.g. MRS and $^2\text{H}_2\text{O}$ (Kunert et al. 2003), further stimulated research on human metabolism and detailed metabolic phenotyping of various populations is now widely used.

Figure 1.1 Number of original papers (dark columns) and reviews (light columns) in five-year intervals from 1970 to 2005 based on MEDLINE searching for the terms ‘diabetes’ or ‘metabolic diseases’.

Despite the rising interest in clinical research, many basic questions regarding metabolism in humans are unanswered or have not been addressed in sufficient detail. A major issue is the role of aging in intermediary metabolism, which was studied in detail *in vivo* in humans only recently. Petersen et al. (2004) assessed glucose fluxes and ectopic lipid deposition as well as mitochondrial oxidation and phosphorylation under *in vivo* conditions and found ~40 % lower flux rates through the tricarboxylic acid cycle and adenosine-tris-phosphate synthesis, possibly explaining the reduced insulin sensitivity and elevated ectopic lipid content in elderly humans. Nevertheless, it remains unclear whether aging is generally associated with impaired insulin sensitivity; specific genetic or even acquired abnormalities could be responsible for reduced mitochondrial function not only during aging but also in other metabolic disorders (Stark & Roden 2007). Likewise, sex and ethnicity can variably affect metabolic characteristics including body fat and its distribution, lipid metabolism and insulin action, which further complicates interpretation of clinical metabolic studies (Woods et al. 2003; Carulli et al. 2005).

These aspects of human metabolism culminate in the recent discussions on definitions of metabolic disorders summarised by the term '(dys)metabolic syndrome' or 'syndrome X', which was introduced by Reaven about 20 years ago (Reaven 1988) and increasingly studied over the next decades (Figure 1.2). The World Health Organisation (WHO), the European Group for Insulin Resistance (EGIR), the Adult Treatment Panel (ATP III) and the International Diabetes Foundation (IDF) re-defined the metabolic syndrome, and more definitions are coming up due to different combinations and cut-off points of continuous variables, such as fasting plasma glucose, triglycerides or variable indices of body fat content (Table 1.1). The current discussion focuses on the relative importance of the compounds of the metabolic syndrome and the issue of whether it is a syndrome at all (Kahn et al. 2005). This controversy mostly results from different interpretations and understandings of this term, which can be used 1) to explain a series of factors by one underlying causal factor, be it insulin

Figure 1.2 Number of original papers (dark columns) and reviews (light columns) in five-year intervals from 1970 to 2005 based on MEDLINE searching for the term 'metabolic syndrome'.

Table 1.1 Definitions of the metabolic syndrome by the World Health Organisation (WHO), the European Group for Insulin resistance (EGIR), the Adult Treatment Panel (ATP III) and the International Diabetes Foundation (IDF). Key: DM – Diabetes mellitus; IGT – impaired glucose tolerance; IFG – impaired fasting glucose

WHO (1999)	EGIR (1999)	ATPIII (2001)	IDF (2005)
<i>Main criteria</i>	<i>Main criterion</i>		<i>Main criterion</i>
Insulin resistance	Insulin resistance		Abdominal obesity
<i>OR</i>			
DM / IGT / IFG			
<i>Other components</i>	<i>Other components</i>	Abdominal obesity	<i>PLUS</i>
Hypertension	Hyperglycaemia	High triglycerides	Hyper-triglyceridaemia
≥ 140/90 mmHg	Hypertension	Low HDL-C	Low HDL-C
Dyslipidaemia	≥ 140/90 mmHg	Hypertension	Hypertension
Central obesity	Dyslipidaemia	≥ 130/85	≥ 130/85 mmHg High
Microalbuminuria	Central obesity	mmHg High	fasting
(two or more)	(two or more)	fasting	glucose/DM
		glucose/DM	(two or more)
		(two or more)	

resistance or obesity; 2) to provide an easy cardiovascular risk cluster in addition to the known traditional risk makers such as cigarette smoking or LDL-cholesterol; or 3) to create a novel clinical syndrome or disease requiring specific treatment. We are currently lacking detailed knowledge of the interaction between numerous polygenic and environmental mediators, variation between populations and ethnic groups and time-dependent changes in these continuous variables. Above all, the physiological basis for the several metabolic disorders remains largely unknown at present.

In addition to its relevance for understanding human pathophysiology, clinical metabolic research is becoming important as a tool in clinical trials on non-pharmacological treatment and even more on drug treatment for today's major epidemiological threats. Of note, searching the MEDLINE detects 580 clinical trials in the 1970s, 1,464 in the 1980s, 5,236 in the 1990s – and already 7,568 between 2000 and 2006.

The sections of this book cover the relevant aspects of current clinical research in humans and are designed to address the researcher's need for theoretical and practical knowledge in this field.

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