Title
Regulation of hepatic intermediary metabolic pathways during HIV infection

Author
Kaempfer, Suzanne Hearne

Publication Date
1990

Peer reviewed|Thesis/dissertation

REGULATION OF HEPATIC INTERMEDIARY METABOLIC PATHWAYS DURING HIV INFECTION

by

Suzanne Hearne Kaempfer

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

NURSING

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

A.N.

The University of California, San Francisco

School of Nursing

REGULATION OF HEPATIC INTERMEDIARY METABOLIC PATHWAYS DURING HIV INFECTION

By

Suzanne Hearne Kaempfer

ABSTRACT

The purpose of this study was to describe regulation of hepatic intermediary metabolism during different stages of HIV infection. Pathways of hepatic glucose utilization during fasting and refeeding were measured using infusions of 1-d1 glucose and acetaminophen (glucuronide probe) in three groups of subjects: healthy volunteers (n=9), asymptomatic HIV infection (n=7), and AIDS (n=7). A method for determining fraction of hepatic gluconeogenesis (GNG) and UDP-gluconeogenesis (UDP-GNG) from phosphoenolpyruvate during fasting using infusions of 13C acetate in conjunction with oral sulfamethoxazole (acetyl probe) and acetaminophen infusions was also tested.

In all groups (N=23) the indirect pathway of hepatic glucose utilization predominated during fasting. Healthy volunteers and asymptomatic HIV subjects tended to switch to the direct glucose pathway with refeeding, while the AIDS group persisted in the indirect pathway. Catabolic subjects with AIDS who were refed by the oral route showed almost no direct hepatic glucose uptake. Large effects of group

membership were observed during fasting and refeeding.

Fraction of hepatic GNG and UDP-GNG were not able to be determined in the limited samples available to the present study due to the error sensitivity of the method employed. Large effects of group membership on isotopic incorporation parameters suggest, however, that group differences in hepatic GNG and UDP-GNG may be detectable in future studies involving larger samples.

Suzanne H. Kaemper
Author

Marilyn J. Dodd
Adviser

ACKNOWLEDGEMENTS

This work was supported in part by an Earle C. Anthony Graduate Dean's Fellowship, University of California, San Francisco, and by National Cancer Institute Predoctoral Traineeship 1 F31 08000.

I would also like to express my gratitude to the members of my thesis committee and the members of Lab 309A for their roles in making the completion of this work possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

The Study Problem

Introduction

Weight loss and wasting of body tissues are frequent sequelae of chronic diseases. Infection with HIV (human immunodeficiency virus) has become a worldwide public health problem, affecting over 500,000 individuals (Sato, Chin, and Mann, 1989). In the U.S. alone over 82,000 cases have been identified (Berkelman, Heyward, Stehr-Green et al., 1989). HIV infection encompasses a spectrum of clinical presentations in a milieu of underlying immune dysfunction, ranging from asymptomatic disease to severe life threatening infectious and nutritional complications. In fact, weight loss and wasting are often prominent clinical features of HIV infection and may precede or coincide with the development of opportunistic infections and malignancies that define progression to AIDS. Thus HIV-associated weight loss is a significant public health concern and clinical problem.

Problem Statement

Weight loss and depletion of body cell mass, indicative of severe, progressive malnutrition commonly occur in patients with AIDS (Acquired Immune Deficiency Syndrome)(Garcia, Collins, & Mansell, 1987; Keithley & Kohn, 1990; Kotler, 1987; Kotler, Tierney, Brenner et al., 1990; Kotler, Tierney, Wang et al., 1989; O'Sullivan, Linke, &

Dalton, 1985). Unfortunately, attempts at repletion of body cell mass by aggressive, expensive, nutritional support interventions have been generally unsuccessful in AIDS patients, many of whom are overtly hypermetabolic due to infectious complications, experience diarrhea and malabsorption, and are anorexic (Hopefl, 1988; Kotler, 1987; Task Force on Nutrition Support in AIDS, 1989). Likewise, for undetermined reasons, clinically stable HIV-infected patients experiencing asymptomatic periods may also remain chronically malnourished and refractory to nutritional interventions (Kotler, Wang, & Pierson, 1985).

Little is known about the underlying pathogenesis of weight loss in HIV infected patients or about factors regulating the progression of HIV infection from asymptomatic disease to AIDS. The design of effective strategies for nutritional rehabilitation and maintenance during HIV infection requires an understanding of the regulation of substrate utilization and how this regulation is affected by the underlying immune deficiency. Given the established adverse effects of malnutrition on immune function, it is conceivable that impaired nutritional status and substrate metabolism could be involved in progression of HIV infection. Consequently, the determination of whether or not defects occur in intermediary metabolism during HIV infection and the relationship of these defects to overt clinical manifestations of HIV infection would

provide potential avenues for developing therapeutic interventions that could prevent disease progression and development of complications.

Significance

Severe weight loss and wasting of lean body tissues often occur during catabolic illness such as that associated with infection or malignancy. Therefore, this complication represents a clinical problem of considerable proportions. Malnutrition associated with catabolic illness affects the course and progression of the disease, the degree of disability experienced by the patient, the ability to respond to therapeutic regimens, and may be the immediate cause of death (Kotler, 1987). Successful assessment and management of weight loss and wasting is thus important to patient survival and morbidity. Most patients with HIV infection who progress to AIDS become profoundly catabolic (Kotler, 1987). This, in conjunction with the magnitude of the public health problem posed by the AIDS epidemic, justifies the selection of HIV infection as a human model in which to study the pathogenesis of weight loss in the context of catabolic illness.

The mechanisms underlying weight loss during catabolic illness are poorly understood. However cytokines (tumor necrosis factor and the interleukins) are likely to be involved, since in animal models they are known to effect a number of changes in metabolism. Such changes include

hyperlipidemia (Feingold, Soued, Staprans et al., 1989; Grunfeld, Wilking, Neese et al., 1989), increased hepatic lipogenesis (Feingold & Grunfeld, 1987), muscle catabolism (Dinarello, 1984a; 1984b; 1988; Flores, Bistrian, Pomposelli et al., 1989), increased hepatic protein content (Fong, Moldawer, Marano et al., 1989), synthesis of acute phase proteins (Dinarello, 1987; 1988), and suppression of adipose lipoprotein lipase activity (Beutler & Cerami, 1985; Dinarello, 1988; Kawakami, Pekala, Lane et al., 1982; Mahoney, Beutler, LeTrang et al., 1985; Semb, Peterson, Tavernier et al., 1987).

Cytokines produced by mononuclear cells have been hypothesized to mediate metabolic disturbances associated with weight loss during HIV infection (Lau & Livesey, 1989; Molina, Scadden, Byrn et al., 1989). Elevated circulating levels of tumor necrosis factor, however, have not been consistently demonstrated in HIV infected subjects (Lahdevirta, Maury, Teppo et al., 1988; Reddy, Sorrell, Lange et al., 1988). Likewise, production of interleukin-1 by cultured peripheral blood monocytes from HIV infected persons occurs (Edelman & Zolla-Pazner, 1989; Weiss, Haeffner-Cavaillon, Laude, et al., 1989), but may be masked by an interleukin-1 inhibitor (Berman, Sandborg, Calabia et al., 1987). Similarly, hypertriglyceridemia unrelated to the degree of wasting has also been observed during HIV infection (Grunfeld, Kotler, Hamadeh et al., 1989).

Accordingly, a better understanding of substrate metabolism during HIV infection must precede further clarification of the association between cytokines and weight loss.

The liver occupies a central role in the regulation of glucose, fat and protein metabolism. Normal adaptation to caloric insufficiency involves mutual inhibition of glucose and fatty acid oxidation in order to spare body protein. Non-invasive probes of hepatic intermediary metabolism provide an opportunity to describe human metabolic regulation in vivo, specifically, the site of metabolic lesions that could potentially contribute to weight loss and wasting. This information may ultimately be used to design therapeutic interventions tailored to specific locations of metabolic dysregulation. Moreover, a better understanding of the inefficacy of current adjuvant nutritional strategies will be possible.

Finally, metabolic abnormalities and resultant cellular dysfunction ultimately appear as clinical problems which are the focus of clinical interventions by nurses and other health professionals. Research on alterations in intermediary metabolism conducted by nurses in a collaborative setting is therefore within the realm of the nursing profession's mission to address actual and potential human responses related to health status.

Research Questions and Purpose

The purpose of this study was to examine regulation of

hepatic intermediary metabolism during HIV infection. The specific aims were: 1) to describe abnormalities in hepatic intermediary metabolism in individuals representing a range of clinical presentations of HIV infection as compared with healthy controls, and 2) to describe abnormalities in hepatic metabolism that are uniquely attributable to the presence of HIV infection.

The research questions to be addressed are: 1) what is the hepatic glucose utilization pathway during fasting and subsequent refeeding in individuals representing a range of HIV infection and weight loss as compared with healthy controls? and 2) what is the nature of hepatic gluconeogenesis and UDP-gluconeogenesis during asymptomatic HIV infection?

Chapter II

Conceptual Framework and Review of Literature

Introduction

The acute phase response to an infectious process (accompanied by anorexia and decreased nutrient intake) elicits a diverse array of metabolic alterations in host protein, fat and carbohydrate metabolism (See Table 1). Normal adaptation to starvation involves mobilization of muscle protein to provide gluconeogenic substrates for brain and anaerobic tissues. Muscle catabolism, however, is regulated in relation to ketogenesis from adipose-derived fatty acids, and the ability of aerobic tissues and the brain to utilize fatty acids and ketones, instead of glucose, as an energy source. During infection, ketogenesis is defective, therefore muscle protein catabolism proceeds unabated to provide glucose precursors. Mobilized protein is also directed into hepatic protein synthesis of acute phase reactant globulins as part of the host defensive response to the infectious agent.

This syndrome of concurrent anabolism and catabolism (reviewed by Beisel, 1972, 1975, 1977a, 1977b; and Powanda, 1977) can be described as an abnormal host response to starvation or caloric insufficiency. Although the mediators of the response to infection are now known to be cytokines, in particular interleukins and tumor necrosis factor released by monocytic cells during phagocytosis of damaged

Table 1

Alterations in Protein, Carbohydrate, and Fat Metabolism during Infection and Inflammation

Protein:

- Increased muscle proteolysis
- Decreased muscle protein synthesis
- Nitrogen wasting/Negative nitrogen balance
- Increased urinary nitrogen loss/Ureagenesis
- Increased hepatic enzyme synthesis
- Decreased hepatic albumin synthesis
- Increased acute phase globulin synthesis
- Increased Branch Chain Amino Acid Oxidation
- Increased plasma phenylalanine/tyrosine ratio
- Increased amino acid flux to liver
- Increased gluconeogenesis from alanine

Carbohydrate:

- Altered glycogen metabolism
- Abnormal glucose tolerance
- Increased glucose (pyruvate) oxidation

Fat:

- Hyperlipidemia
- Increased hepatic lipogenesis
- Inhibition of physiologic ketosis
- Decreased peripheral lipid disposal (decreased lipoprotein lipase activity)

tissue or pathogenic organisms (Cannon, Tompkins, Gelfand, et al., 1990; Dinarello, 1984a,1984b, 1987, 1988; Girardin, Grau, Dayer et al., 1988; Grau, Taylor, Molyneux et al., 1989; Kern, Hemmer, VanDamme et al., 1989; Le & Vilcek, 1987; Liao & Rosenstreich, 1983; Michie, Manogue, Spriggs et al., 1988; Mizel, 1989), the underlying mechanisms and specific metabolic pathways involved are poorly understood.

Much of the research on the metabolic effects of infection has employed both human and animal models of experimentally-induced bacterial, viral, and parasitic infection. Originally this work was performed in conjunction with the development of diagnostic methods and vaccines. More recently, host responses to infection have been studied within the context of sepsis during surgery, trauma, burns, or tissue infarction (Kushner, 1982), confounding the distinction between the unique effects of infection and inflammation, and the combined effects of injury, infection, starvation, immobility, electrolyte imbalance, and shock ("septic post-traumatic catabolism")(Border, 1970; Goldstein & Elwyn, 1989; Ryan, 1976; Wilmore, 1976). With the emergence of HIV infection as a significant cause of wasting, interest in the effects of chronic infection (as distinct from infection accompanying surgery or trauma) on metabolism has been rekindled (Berman, Sandborg, Calabia et al, 1987;

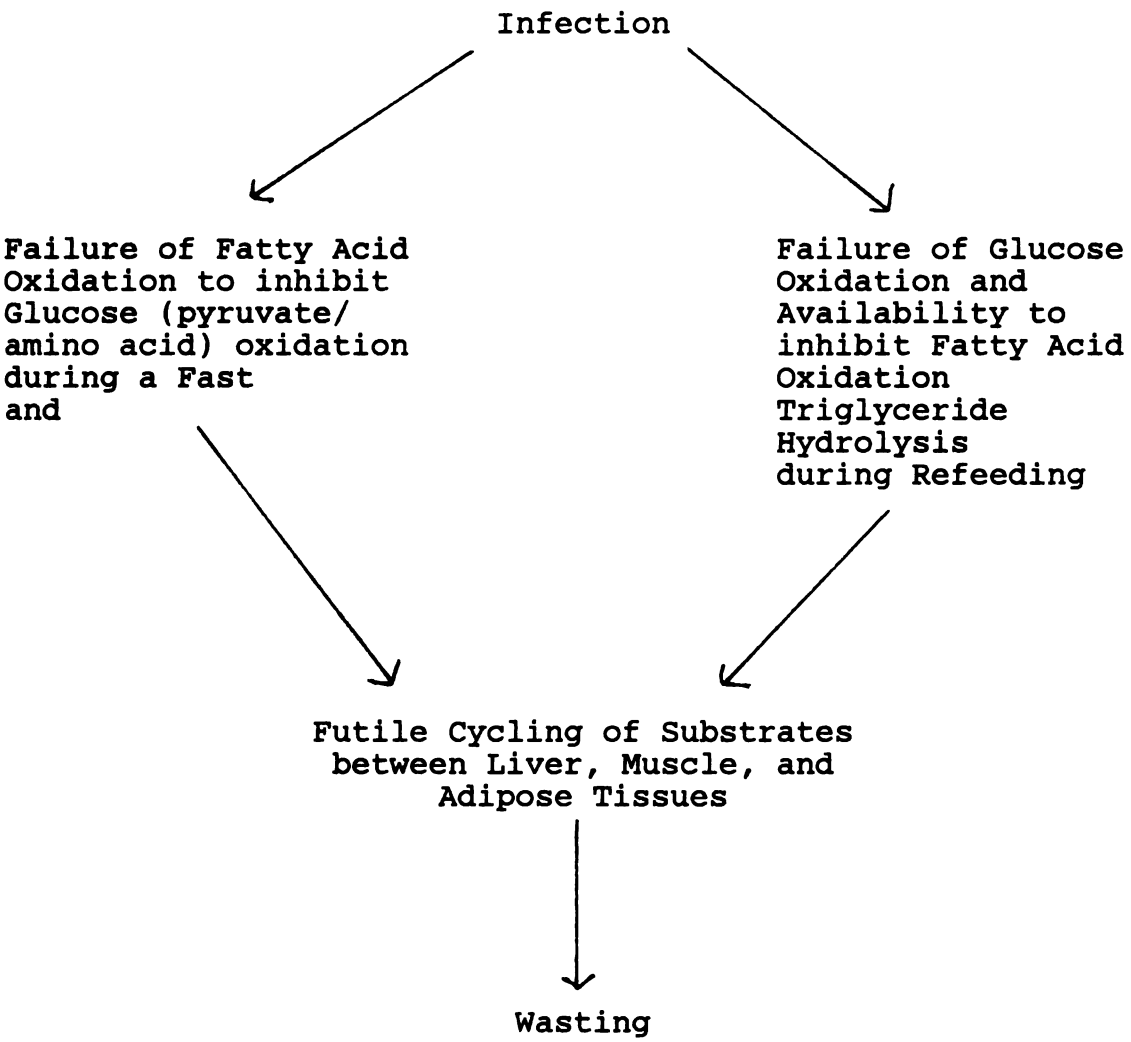
Chlebowski, 1985; Kotler, 1987; Kotler, Wang, & Pierson, 1985).

The clinical manifestations of starvation in man have been amply described (Cahill, 1970; Saudek & Felig, 1976; Viteri, 1981), and from the perspective of metabolic regulation, are explained by the interaction between carbohydrate and fat metabolism. Simply stated, during caloric insufficiency, fatty acid and ketone oxidation inhibit glucose oxidation and preserve protein. Conversely, refeeding suppresses fatty acid and ketone oxidation by allowing glucose utilization and oxidation, and lipogenesis to proceed (Newsholme & Start, 1973; Newsholme & Leech, 1983; Randle, Garland, Hales et al., 1963). The net effect of these processes is the preservation of body protein through the utilization of fatty acids and ketones instead of glucose (pyruvate) during periods of starvation. Failure of fatty acid utilization to inhibit glucose oxidation would result in use of body protein (with enhanced gluconeogenesis and ureagenesis) as a fuel source during fasting. Failure of glucose utilization to inhibit fatty acid oxidation (in conjunction with fatty acid synthesis) and triglyceride hydrolysis (in conjunction with re-esterification) during refeeding would result in futile cycling (See Figure 1).

The liver occupies a central position in this process as a consequence of its unique properties. These properties, which can be exploited to monitor fuel flow

Figure 1

Model of Failure of Mutual Inhibition of Fatty Acid and Glucose Oxidation during Infection



include (Katz & McGarry, 1984; McGarry, Kuwajima, Newgard et al., 1987):

1) possession of complete enzyme systems for synthesis and catabolism of glucose, glycogen and fat,

2) ability to respond to nutritional and endocrine signals by changing the direction of carbon flow through pathways of lipid and carbohydrate metabolism,

3) principal site of ketogenesis, ureagenesis, triglyceride synthesis (VLDL), glucose synthesis, and detoxification of compounds (conjugation) for excretion.

The metabolic sequelae of infection have never been studied or described explicitly in terms of failure of the mutual inhibition of glucose and fatty acid oxidation. This is because previously available methods have not permitted the continuous in vivo monitoring of metabolic fluxes, as well as the relative contributions of liver, muscle, and adipose tissues to those fluxes during fasting and refeeding.

A recent innovation in the study of hepatic substrate metabolism has involved using a combination of isotope tracer methods, metabolic end product techniques, and non-invasive xenobiotic probes. These enable the determination of fluxes of glucose and fatty acid carbon through the major hepatic metabolic pathways, the activity of regulatory enzymes involved, and thereby the integration between pathways involved in the mutual inhibition between glucose

and fatty acid oxidation (Hellerstein, 1988b). Specifically, two non-invasive xenobiotic probes, the glucuronide probe and the acetyl probe, have been recently developed and provide access to two hepatic intracellular metabolites (UDP-glucose and acetyl-CoA, respectively) (Hellerstein, Greenblatt, & Munro, 1986; 1987; Hellerstein & Munro, 1987; 1988; Hellerstein, 1989; Kaempfer, Wu, & Hellerstein, 1989). Used in conjunction with isotope tracer methods, non-invasive probes permit the testing of regulation of intracellular metabolic pathways in vivo, and provide a conceptual framework within which to study potential defects in mutual inhibition of glucose and fatty acid oxidation in a human model of infection, HIV infection.

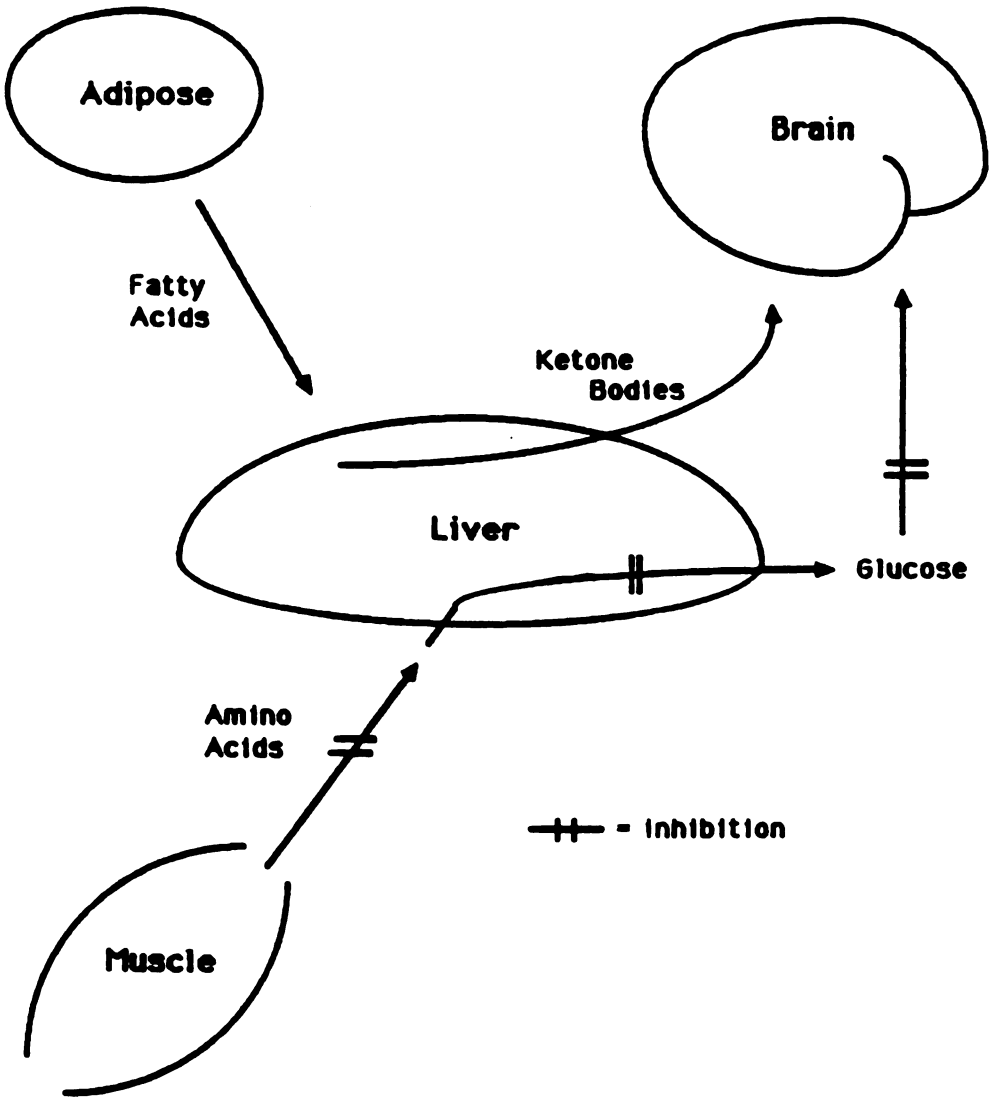
The Normal Metabolic Response to Starvation

The integration of metabolic fuels during the phases of starvation in the intact organism is depicted in Figure 2 (Goldberg & Chang, 1978; McGarry & Foster, 1980; Newsholme & Start, 1973; Newsholme & Leech, 1983; Ruderman, 1975). Starvation begins with the post-absorptive phase. After the contents of the previous meal have been absorbed, liver glycogenolysis provides glucose for peripheral tissues. This phase lasts several hours, with glucose oxidation predominating.

The post-absorptive phase is followed by early starvation (until 24 hours after the previous meal). As liver glycogen stores decrease and insulin levels drop,

Figure 2

Integration of the Normal Metabolic Adaptation to Starvation



fatty acids are mobilized from adipose tissue and their rate of oxidation by muscle and other tissues increases, replacing glucose utilization and oxidation. Liver glycogen continues to provide glucose mainly for brain and anaerobic tissues; hepatic ketogenesis begins.

The phase of intermediate starvation lasts from 24 hours to several weeks after the previous meal. During this phase, hepatic glycogen becomes depleted, therefore glucose is provided by hepatic gluconeogenesis from mobilized muscle amino acids (especially alanine), glycerol (from mobilized adipose triglyceride), and lactate (from anaerobic glycolysis). (The kidney performs gluconeogenesis from glutamine to a lesser extent.) Fatty acids continue to be used as a fuel source by muscle and other tissues. However, as ketone concentration in the blood increases, muscle and other tissues begin to use ketones as an additional fuel source, and the brain begins to use ketones in addition to glucose. During intermediate starvation, ketones regulate the rate of adipose fatty acid mobilization in relation to tissue energy needs by directly inhibiting lipolysis, and by increasing the sensitivity of adipose tissue to the antilipolytic effects of insulin.

The phase of prolonged starvation lasts from intermediate starvation until refeeding or death. Muscle tissue gradually reduces its use of ketones, relying predominantly on fatty acids for fuel. The increase in

plasma ketone concentration (physiologic ketosis) promotes a shift by brain from predominantly glucose to predominantly ketone oxidation. This decreases the demand for glucose from gluconeogenesis, thus suppressing muscle amino acid mobilization.

From in vitro studies the mechanisms whereby fatty acid and ketone oxidation suppress glucose oxidation during a fast may in part be explained by a reduction of flux through glycolysis and in part by reduced pyruvate dehydrogenase (PDH) activity. First, beta oxidation of fatty acids increases acetyl CoA concentration and yields NADH (reduced nicotinamide adenine dinucleotide). Increased NADH and increased ratio of acetyl CoA to CoA inhibits PDH by favoring conversion to its inactive form through end product inhibition. This inhibits pyruvate oxidation, thus preserving glucose and glycogen. In the liver available oxaloacetate is used for gluconeogenesis (instead of condensing with acetyl CoA to form citrate for lipogenesis), thus allowing ketogenesis from acetyl CoA to proceed. In peripheral tissues, acetyl CoA from beta oxidation or ketone oxidation is used for citrate synthesis prior to oxidation to CO2. Citrate inhibits phosphofructokinase and thus flux through glycolysis. Finally, as glucose-6-phosphate accumulates, glucokinase in liver or hexokinase in the periphery (and therefore glucose utilization) are inhibited, in favor of fatty acid and ketone utilization. These

mechanisms are reversed with refeeding. The normal metabolic adaptation to starvation by the liver is depicted in Figure 3 and provides the conceptual basis for the present study.

Conceptual Framework for Studying The Normal Metabolic Response to Starvation

The conceptual framework for the present study approaches hepatic substrate regulation during infection from the perspective of failure of mutual inhibition of glucose and fatty acid oxidation, and employs a methodology based on an adaptation of metabolic end product analysis, the precursor-product relationship between metabolites in metabolic pathways, isotope dilution principles, and the physiology of the direct and indirect pathways of hepatic glucose utilization. Selected parameters in pathways of hepatic glucose and fatty acid utilization are monitored *in vivo* during fasting and refeeding. From isotopic labeling patterns of precursors and products, fluxes through these pathways can be determined and enzyme regulation inferred.

Figures 4 and 5 depict pathways of hepatic glucose and fatty acid utilization. Hepatic glucose utilization parameters that can be monitored using this methodology include:

1) plasma glucose (derived from exogenous glucose or from gluconeogenesis from recycled trioses),

2) UDP-glucose (the precursor of glycogen and

Figure 3

The Normal Metabolic Adaptation to Starvation by the Liver

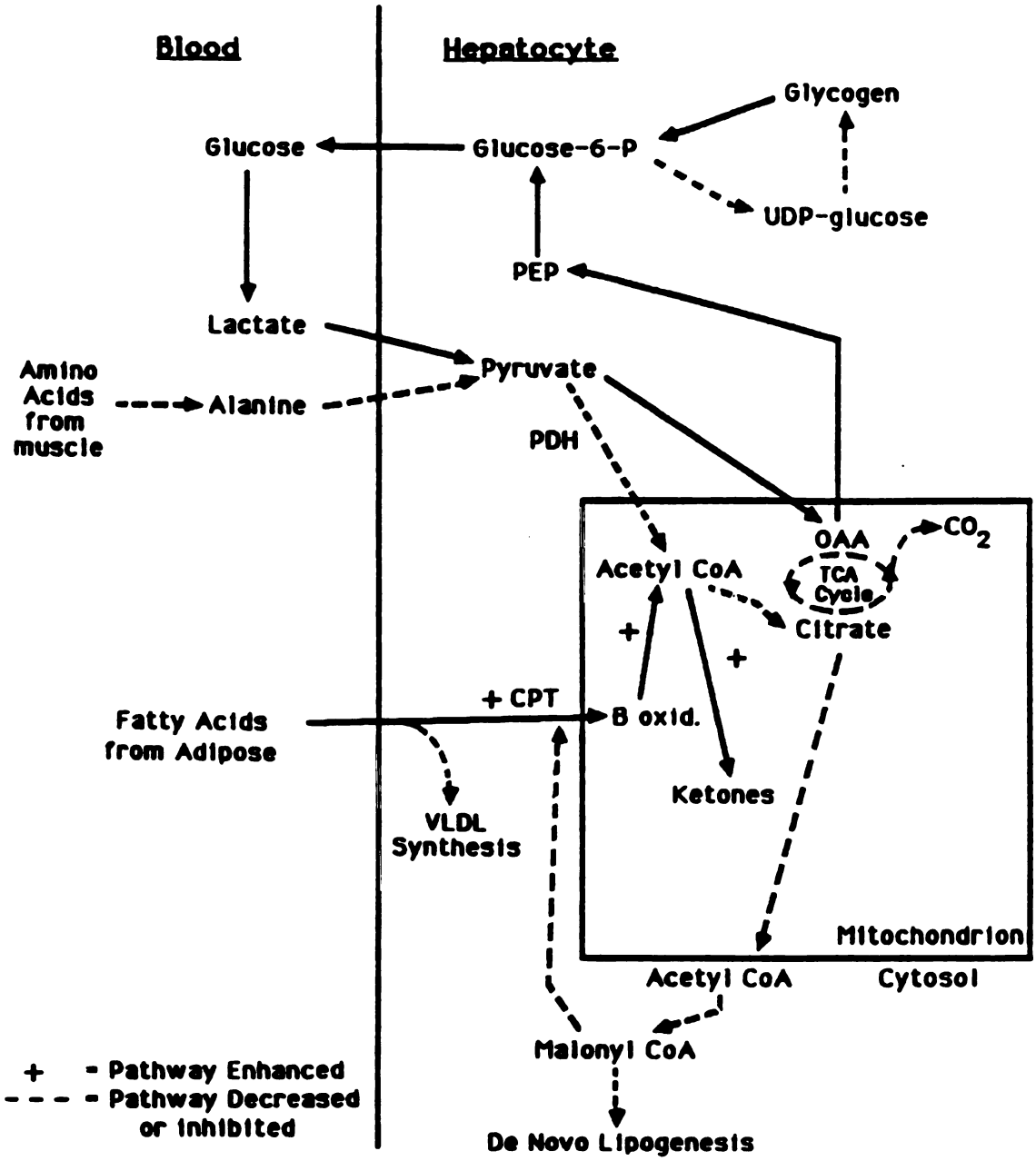
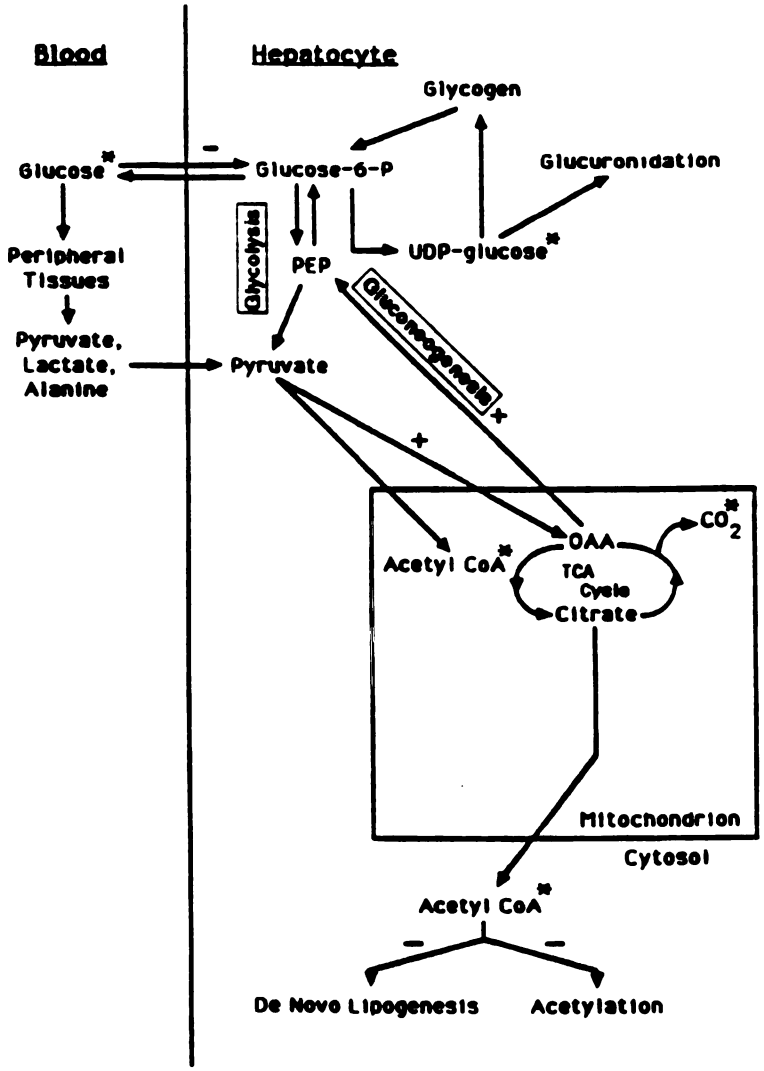


Figure 4

Pathways of Hepatic Glucose Utilization



*Designates a parameter monitored using non-invasive xenobiotic probes in conjunction with tracer methods

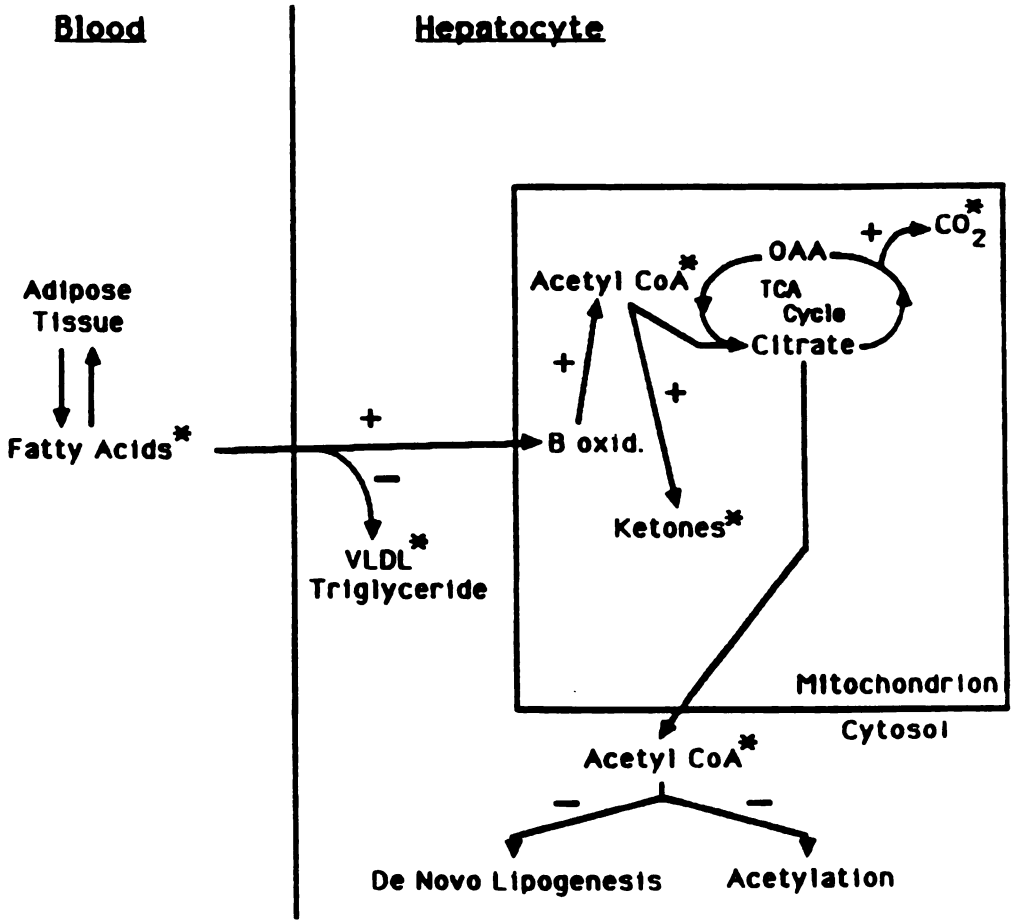
+Pathways activated during a fast (reversed during refeeding)

-Pathways inactivated during a fast (reversed during refeeding)

Note. Adapted from Substrate pathways, monokines, wasting, and HIV infection by M. K. Hellerstein, 1988. Reprinted by permission.

Figure 5

Pathways of Hepatic Fatty Acid Utilization



*Designates a parameter monitored using non-invasive xenobiotic probes in conjunction with tracer methods

+Pathways activated during a fast (reversed during refeeding)

-Pathways inactivated during a fast (reversed during refeeding)

Note. Adapted from Substrate pathways, monokines, wasting and HIV Infection by M. K. Hellerstein, 1988. Adapted by permission.

glucuronidation reactions),

3) mitochondrial acetyl-CoA (the product of pyruvate oxidation and, hence, an indicator of PDH, pyruvate dehydrogenase, activity),

4) CO2 (from oxidation of pyruvate-derived mitochondrial acetyl-CoA),

5) cytosolic acetyl-CoA (from pyruvate-derived mitochondrial acetyl-CoA, destined for fatty acid/ VLDL triglyceride synthesis and acetylation reactions).

Hepatic fatty acid utilization parameters that can be monitored include:

1) VLDL triglyceride (synthesized directly from fatty acids),

2) mitochondrial acetyl-CoA (derived from beta oxidation of fatty acids),

3) ketones (via mitochondrial acetyl-CoA from partial oxidation of fatty acids in mitochondria),

4) CO2 (from oxidation of fatty acid derived mitochondrial acetyl-CoA),

5) cytosolic acetyl-CoA (from fatty acid-derived mitochondrial acetyl-CoA and destined for fatty acid/ VLDL triglyceride re-synthesis and acetylation reactions).

In terms of the parameters described above, normal metabolic adaptation to a fast, implying predominance of fatty acid oxidation, would be indicated by (Figures 4 and 5):

1) greater production of acetyl-CoA from fatty acid than from glucose precursors,
2) greater production of UDP-glucose by recycled glucose (via trioses) than nonrecycled glucose (direct hepatic uptake),
3) reduced synthesis of acetyl-CoA (and therefore fatty acid/ triglyceride) from glucose,
4) oxidation rather than re-esterification (to triglyceride) of fatty acids.

During refeeding, normal metabolic adaptation, implying predominance of glucose (pyruvate) oxidation, would be indicated by (Figures 4 and 5):
1) greater production of acetyl-CoA from glucose than from fatty acid precursors,
2) relatively increased production of UDP-glucose by nonrecycled glucose than recycled glucose,
3) increased de novo lipogenesis from glucose precursors,
4) inhibition of beta-oxidation of fatty acids and ketogenesis,
5) increased re-esterification of fatty acids to triglyceride.

The foregoing conceptualization of the metabolic adaptation to starvation has never been validated in either an animal or human model of infection. Several aspects of carbohydrate and fat metabolism as described, however, have undergone testing in an animal model and preliminary testing

in humans is in progress (described below).

Following a brief overview of basic principles of isotope tracer methods and end product analysis employed in these techniques, the methods of applying the glucuronide probe, the acetyl probe, and stable isotopes to the study of regulation of hepatic intermediary metabolism during HIV infection will be presented. Specific parameters to be described are:

1) rate of appearance and labeling pattern of UDP-glucose,

2) rate of appearance and labeling pattern of plasma glucose,

3) rate of appearance and labeling pattern of hepatic cytosolic acetyl-CoA

4) gluconeogenesis

5) UDP gluconeogenesis.

Tracer Methodology

The continuous formation (synthesis) and utilization (breakdown) of metabolites in an organism is called turnover (Hetenyi, Perez, & Vranic, 1983). Turnover describes a process of replacement of a substance, therefore it may involve production of new molecules with replacement of molecules already present, or it may involve exchange of material with the same material in another site (Waterlow, Garlick, & Millward, 1978).

Because metabolites are released by cells into the circulation for use by other cells or for excretion, flux or

flow of a metabolite through the circulation represents systemic utilization. The rate of appearance of a metabolite is defined as the overall rate of its release (from whatever source, i.e. cells or exogenous sources) into the circulation (or into an intracellular pool). Rate of disappearance is the rate at which a metabolite is used by cells or excreted, thus leaving the circulation (or intracellular pool)(Hetenyi, Perez, & Vranic, 1983).

The amount of a metabolite in the circulation (or any other site accessible to sampling) is its pool. In a physiologic steady state (such as in the post-absorptive state at rest), the rates of appearance and disappearance are equal, the pool size does not change with time, and the volume in which the pool is dissolved is constant. Therefore steady state is recognized as a stable concentration of a metabolite in plasma (or in an intracellular pool). During a steady state, turnover is equivalent to both rate of appearance and rate of disappearance (Hetenyi, Perez, & Vranic, 1983; Wolfe, 1984).

Isotopic tracer methods label metabolites in a pool and are employed to determine the turnover of a metabolite. (The present discussion will be limited to continuous infusion tracer methods.) Atoms of the metabolite of interest are labeled with isotopes (radioactive or stable), then the metabolite is injected into the circulation in an amount that is detectable but does not interfere with normal

metabolism (i.e. a tracer quantity). It is assumed that the labeled metabolite is not discriminated from unlabeled metabolite and therefore that the label traces the movement of unlabeled as well as labeled molecules. Isotopic equilibrium or plateau is achieved when the concentration of labeled and unlabeled metabolites in plasma attains a constant value. At isotopic equilibrium, there is a plateau in isotopic enrichment (relative abundance of isotope) determined by serial measurements. Thus isotopic steady state is defined as constant isotopic enrichment of a labeled pool over time (Wolfe, 1984). Using tracers, the rate of appearance or turnover is the rate at which unlabeled metabolite enters the circulation, i.e. the dilution of labeled by unlabeled metabolite, referred to as isotopic dilution (Hetenyi, Perez, & Vranic, 1983; Wolfe, 1984).

Application of tracer methods to determine turnover, therefore, involves infusion of tracer into the circulation, in order to achieve an isotopic steady state, sampling of plasma or other pools at intervals, and determination of the concentration of labeled and unlabeled metabolite. The ratio of labeled to unlabeled metabolite is referred to as its specific activity (using radioactive isotopes) or enrichment (using stable isotopes). Turnover (in units of mass per unit of time, e.g. ug/hour) can be calculated from the rate of tracer infusion and specific activity or

enrichment in a metabolic pool as (Hetenyi, Perez, & Vranic, 1983; Wolfe, 1984):

$$\text{turnover} = \frac{\text{infusion rate of tracer}}{\text{specific activity or enrichment}}$$

This calculation, however, involves the following assumptions:

1) steady state conditions as previously described,

2) constant rate of appearance,

3) random movement of molecules, i.e. random appearance and disappearance of both labeled and unlabeled molecules (Zilversmit, Entenman, & Fishler, 1943).

Rapid turnover means the specific activity or enrichment is low (the label is more dilute due to rapid influx of unlabeled metabolite). Slow turnover means high specific activity or enrichment (label concentrated due to slow influx of unlabeled metabolite). Thus turnover is not directly measured, but calculated from measurements of behavior of labeled and unlabeled metabolites in plasma or other pools. The validity of this calculation depends on the validity of the metabolic model selected to represent the kinetics of the selected metabolite (Waterlow, Garlick, & Millward, 1978).

The labeled atoms of a tracer can be reincorporated into molecules of a metabolite after leaving the circulation, then subsequently reappear in the plasma. This is called recycling. When recycling of a metabolite occurs

(as with glucose, which is discussed later), calculations of turnover will underestimate the true rate of turnover. To overcome this, a label that does not recycle can be selected instead (such as tritium or deuterium, which are converted to water during metabolism), although the position of the labeled atom in the molecule can also influence the calculated value of turnover (Hetenyi, Perez, & Vranic, 1983).

The Precursor-Product Relationship

Tracer methods are applied to metabolic models which describe the physiological processes in which a metabolite is involved and provide a framework for quantitatively analyzing those processes (Waterlow, Garlick, & Millward, 1978). Most metabolic models assume that metabolite pools are in steady state, that exchanged or newly-introduced molecules mix completely and instantaneously in the metabolite pools, and that transfer or exchange between pools occurs in a constant fraction per unit time (based on the concept of randomness, i.e. that there is no selection between labeled and unlabeled molecules).

Quantitative determination of rates of transfer between metabolites in metabolic pathways can thus be determined by applying tracer methods to metabolic models (Hetenyi, Perez, & Vranic, 1983). Label is introduced into a precursor pool and the appearance of label in a metabolic product of that precursor is monitored. During steady state conditions when

the specific activity of the precursor reaches a plateau, the product specific activity also plateaus. As a result, the relationship between precursor and product can be expressed as a ratio, product specific activity/ precursor specific activity. This ratio indicates the fraction of the product arising from the precursor. (The precursor-product relationship between two metabolites in a pathway can also be used to determine the rate of synthesis of product from precursor (Waterlow, Garlick, & Millward, 1978). Implicit in this formulation is the assumption that the labeled precursor pool is the only source of label in the product pool (Hetenyi, Perez, & Vranic, 1983). If labeled precursor molecules are able to exchange label with other molecules prior to entering the product, calculations of rates of transfer will be underestimated.

End Product Analysis

Traditionally, two major problems have attended attempts to infer regulation of enzymes in metabolic pathways by following metabolic fluxes in intact organisms (Hellerstein, 1988a): the difficulty of accessing intracellular metabolic pools *in vivo*, and the inability to access immediate precursors, except by *in vitro* methods. These problems, however, may be overcome by an adaptation of end product analysis used in conjunction with tracer methods.

End product analysis as described by Waterlow, Garlick,

& Millward (1978), is based on the precursor-product relationship. In a metabolic pathway, the immediate precursor of a compound is the last "stable" compound preceding it (although a compound may have more than one immediate precursor)(Zilversmit, Entenman, & Fishler, 1943). Because both labeled and unlabeled molecules must enter the product exclusively by way of the immediate precursor, the specific activities of product and precursor are identical at a given point in time. (Thus the precursor-product relationship as defined by end product analysis obtains during both steady state and non-steady state conditions.)

The prototype method of end product analysis was the arginine-urea method of sampling an intracellular hepatic amino acid pool (arginine) in vivo (Reeve, Pearson, & Martz, 1963; Swick, 1958). Labeled CO2 (precursor) is infused and incorporated into the guanidine-carbon of arginine during the urea cycle. The labeled urea (product) is cleaved, thus providing a measure of the rate of appearance of free intracellular hepatic arginine. A major problem of this method, however, is that the transfer-RNA-amino-acyl conjugate, not the free amino acid in a cell, is the true precursor for protein synthesis. Therefore, access to free arginine can not be truly representative of intracellular amino acid pools used in protein synthesis (Golden & Waterlow, 1977). Two recent adaptations of end product analysis for hepatic glucose and fatty acid metabolites

avoid the problem of direct access to true precursor pools: the glucuronide probe and the acetyl probe.

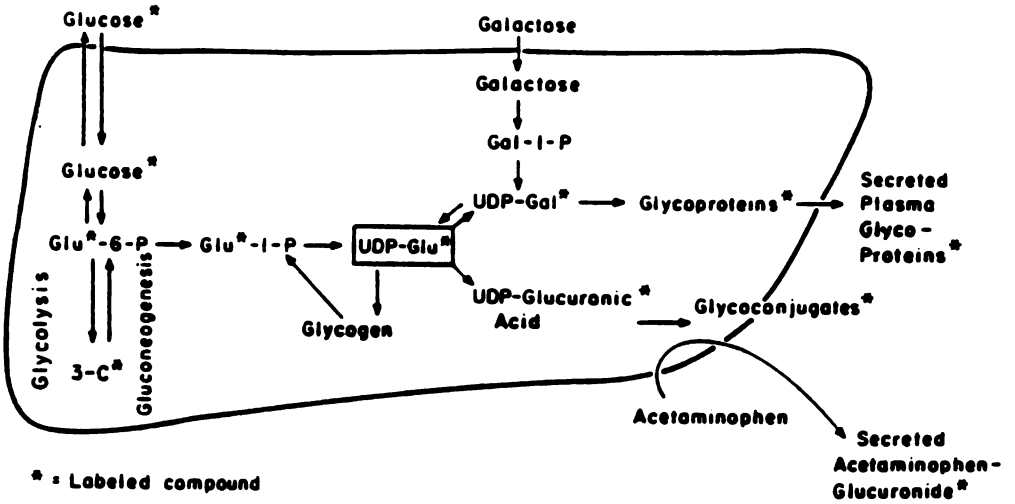
The galactose-glucuronide method (glucuronide probe) was developed by Hellerstein (1986). Analogous to the arginine-urea method, a labeled precursor (e.g. glucose) enters an intracellular hepatic metabolite (the hexose of UDP-glucose). The UDP-glucose (true precursor) is partitioned into UDP-galactose and UDP-glucuronic acid, and subsequently into secreted metabolites, plasma glycoproteins and glucuronyl-conjugates, respectively. (See Figure 6). The present discussion will be limited to the glucuronyl-conjugate pathway.

UDP-glucuronic acid comes exclusively from UDP-glucose and is the immediate precursor for secreted glucuronidated xenobiotics, e.g. acetaminophen. Therefore, the specific activity of newly synthesized acetaminophen-glucuronide is the same as that of UDP-glucose at any given point in time. Acetaminophen-glucuronide is rapidly secreted into the bloodstream and excreted in the urine. Thus acetaminophen-glucuronide provides access to intracellular hepatic UDP-glucose pools and thereby, access to fluxes of hepatic intracellular nucleotide sugar pools <u>in vivo</u>.

The acetyl probe evolved from the carbohydrate moiety labeling method (Kaempfer, Wu, & Hellerstein, 1989). Its utility derives from the central role of acetyl-CoA in metabolism and the access to hepatocellular acetyl-CoA

Figure 6

Schematic Model of Hepatic Hexose and UDP-Sugar Metabolic Pathways



Note. From Glycoconjugates as non-invasive probes of intrahepatic metabolism by M. K. Hellerstein, 1986. Ann Arbor, MI: University Microfilms International. Copyright 1986 by M. K. Hellerstein. Reprinted by permission.

afforded by the occurrence of acetylation reactions in hepatic parenchymal cells (Hellerstein & Kaempfer, unpublished observations, 1989; Morland & Olsen, 1977; Olsen, 1982; Olsen & Morland, 1978; 1983). Like glucuronidation, acetylation is a means whereby xenobiotics, such as sulfa drugs, are conjugated for excretion (Hartiala, 1973; Sigel, 1983).

The acetyl probe involves the administration of the xenobiotic sulfamethoxazole along with labeled precursors (e.g. glucose or acetate). The label enters the intracellular hepatic metabolite, acetyl CoA, which is partitioned into ketones, fatty acids/triglyceride, CO2, or acetylation reactions. Acetyl CoA also contributes carbon to glucose and UDP glucose synthesis (to be discussed later) through metabolic exchange with oxaloacetate (Brosnan, 1982; Consoli, Kennedy, Miles et al., 1987; Hetenyi, 1982; Katz, 1985; Krebs, Hems, Weidemann et al., 1966; Strisower, Kohler, & Chaikoff, 1952; Weinman, Strisower, & Chaikoff, 1957). Thus, sulfamethoxazole-acetate, the acetylated conjugate of sulfamethoxazole, which is excreted into plasma and then urine, reflects intracellular hepatic acetyl-CoA pools (its immediate precursor) and can be used to monitor fluxes of the products of acetyl-CoA (See Figure 7).

The glucuronide and acetyl probes represent non-invasive metabolic probes of hepatic intracellular metabolites. (See Table 2). As such, several assumptions

Figure 7

Structure of Sulfamethoxazole Acetate

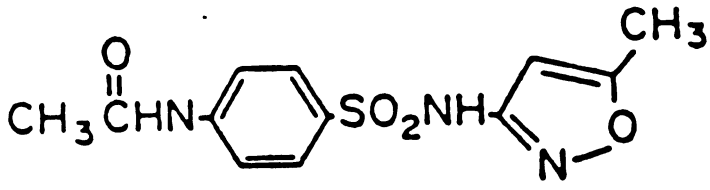


Table 2

Characteristics of Noninvasive Metabolic Probes

-Metabolic conjugates that are synthesized exclusively or nearly exclusively by the liver

-Secreted into blood and excreted in urine without storage or complex kinetics

-Readily isolated from blood or urine

-Given with particular labelled precursors whose behavior in hepatic metabolic pathways is characterized

Note. From "New non-invasive stable isotopic probes for studying regulation of hepatic metabolic pathways in man" by M. K. Hellerstein, 1988a. Reprinted by permission.

are made in using them for end product analysis to determine metabolic fluxes in vivo (See Table 3). These include (Hellerstein, 1986; Sigel, 1983):

1) the kinetics of the secreted end product in plasma should not be complex (such as delayed urinary excretion, multiple tissue storage pools, or reutilization through other pools),

2) the immediate precursor and product should be located in the same hepatocyte associated with the same subcellular fraction,

3) non-steady state physiological conditions (i.e. feeding state or direction of glycolytic flux) should not alter the isotopic relationship between precursor and product.

Use of Non-Invasive Probes to Monitor Mutual Inhibition of Glucose and Fatty Acid Oxidation

In order to determine rate of appearance or isotopic labelling patterns of hepatic UDP-glucose or acetyl-CoA, label is infused at a constant rate into plasma with subsequent labeling of UDP-glucose and acetyl-CoA pools. UDP-glucose is readily labeled by both glucose and galactose tracers because most exogenous galactose is metabolized via hepatic UDP-glucose in mammals, and because much of UDP-glucose is ultimately derived from plasma glucose. (See Figure 6). Acetyl-CoA is readily labeled using glucose tracers via PDH activation (Blackham, Kaempfer, Wu et al., 1990) and using acetate tracers because in mammalian liver, acetate thiokinase, the enzyme which converts acetate

Table 3

Requirements for Use of End-Product Analysis

-End-product is derived from the metabolite under consideration, in the same compartment of the same cell
-End-product is derived exclusively from the metabolite under consideration (no other pathways exist for entry of labeled or unlabeled molecules)
-No functional compartmentalization exists within metabolite or end-product pools
-Newly formed end-product is accessible to sampling in vivo (no time lag of appearance and no tissue storage pools)
-Metabolite and end-product can be labeled using available tracers in reasonable quantities
-Varying of physiologic or experimental conditions should not alter the relationship

Note. From Glycoconjugates as non-invasive probes of intrahepatic metabolism by M. K. Hellerstein, 1986, Ann Arbor, MI: University Microfilms International.

to acetyl CoA, is predominantly cytosolic (making it readily available for acetylation reactions) (Ballard, 1972; Lundquist, 1962). Both the xenobiotic probe and the label are administered for a sufficient period of time to permit the attainment of isotopic plateau in the selected probe. At that time, the standard turnover (or rate of appearance) equation can be applied.

A number of studies using the glucuronide and acetyl probes have already demonstrated their use in studying regulation of carbohydrate and fat metabolism in both animals and humans. Initially, the relative contributions of the direct and indirect pathways of glucose utilization to hepatic UDP-glucose were determined in rats. The significance of this application of the glucuronide probe may be better appreciated by considering the physiology of hepatic glucose utilization.

A growing body of literature suggests that glucose is a poor substrate for liver metabolism (reviewed by Katz & McGarry, 1984; McGarry, Kuwajima, Newgard et al., 1987). Instead, during both fasting and feeding, glucose carbon enters hepatic metabolic pathways through recycled (indirect) pathways. Recycling means that glucose is glycolyzed presumably in peripheral tissues, to trioses (pyruvate, lactate, alanine), which then return to the liver at the level of pyruvate, destined for glycogen synthesis, gluconeogenesis, or lipogenesis (depending on feeding state

and enzyme activation). (See Figures 3 and 5). Direct (non-recycled) uptake of glucose by the liver occurs, but only during late refeeding, and it is quantitatively less important than the indirect pathway (McGarry, Kuwajima, Newgard et al., 1987). This apparently physiologic futile cycle has been referred to as the "glucose paradox" because net glucose carbon must enter hepatic metabolic pathways (for glycogen and fat synthesis) but the glucose molecule itself is not utilized effectively by the liver.

Glucose labeled in position one with deuterium or tritium (isotopes of hydrogen) is particularly suited for studies of recycling because the label is lost during passage through three and four carbon metabolic pools (representing metabolism of glucose to the level of trioses) (Katz & Rognstad, 1976). Using two labels of glucose (carbon and hydrogen) in conjunction with the glucuronide probe, the pathway by which glucose enters UDP-glucose would be direct if both labels were retained equally in excreted acetaminophen-glucuronide (i.e. a ratio of 1 compared to the ratio in the infused glucose). The indirect pathway would be indicated by the preferential loss of deuterium or tritium relative to carbon label, yielding an isotopic ratio of less than one in the excreted glycoconjugate. Thus a ratio of less than one would indicate the extent to which glucose traversed the hepatic gluconeogenic pathway before entering UDP glucose.

Using the glucuronide probe with infusions of 1-3H- and U-14C-glucose, Hellerstein (1986; Hellerstein, Greenblatt, & Munro, 1986) found that during fasting and the first few hours of refeeding, the recycling pathway predominated, accounting for 64% of hepatic UDP-glucose synthesis. Late in refeeding (after 3-5 hours), the fraction of hepatic UDP-glucose synthesized by the direct pathway increased to 60-70% and this fraction was significantly correlated with degree of glycogen repletion (R2=0.68, p<.0001). In parallel studies in normal human volunteers involving infusion of 1-d1 glucose with the glucuronide probe, recycling of glucose was found to contribute 60-80% of UDP-glucose during fasting (Hellerstein, Wu, Kaempfer et al., 1989) With refeeding 42-57% of UDP-glucose was derived by the direct pathway. In these studies, enrichment of UDP-glucose was compared to enrichment of plasma glucose to determine direct versus indirect pathway (i.e. a ratio of 1 would imply direct, while a ratio of less than 1 would imply indirect pathway). (This is analogous to comparing the ratio of 1-d1- to U-13C- glucose enrichment between feeding states.)

In a related study using the same methods, rats were given dichloroacetate (an activator of hepatic PDH) during refeeding (Hellerstein, Xie, & Munro, 1987). Liver glycogen deposition was prevented and the 3H/14C acetaminophen-glucuronide ratio rose from 0.38 to 0.65 (indicating a

decrease in glucose recycling from 62 to 35%), consistent with activation of hepatic PDH. Thus PDH activation appeared to correlate with suppression of synthesis of hepatic UDP-glucose (and therefore glycogen) from trioses.

The rate of appearance of hepatic UDP-glucose has been determined using 1-3H galactose and the glucuronide probe in rats (Hellerstein, 1989). Mean fasting rate of appearance of UDP-glucose was 4.5 mg./kg./min. Within 3-4 hours after refeeding, acetaminophen glucuronide specific activities fell and mean rate of appearance of UDP-glucose increased to 13.1 mg./kg./min. Glycogen deposition was correlated with the increase in rate of appearance of UDP-glucose from fasted values (R2=0.763, p<.001). The rate of appearance of hepatic UDP-glucose was also determined in normal human volunteers using 1-d1 galactose (Hellerstein, Wu, Kaempfer et al., 1989). Mean fasting rate of appearance of UDP-glucose (0.97-1.07 mg./kg./min.) rose to 1.75-2.07 mg./kg./min. with refeeding, congruent with the findings in rats.

The acetyl probe has also been tested in rats and in humans. Rats infused with either 1- or 2-14C acetate and sulfamethoxazole demonstrated fasting sulfamethoxazole acetate specific activities of 37-58 dpm/ug (Kaempfer, Wu, & Hellerstein, 1989; Wu, Kaempfer, Reid et al., 1989). With refeeding, the specific activities fell to 17-31 dpm/ug, indicating dilution of acetyl CoA with unlabeled acetyl

units, most likely through activation of PDH. Likewise in normal human volunteers receiving 1-13C acetate with the acetyl probe, fasting enrichments of sulfamethoxazole acetate fell with refeeding, with rate of appearance of sulfamethoxazole acetate increasing a mean of 51% (Kaempfer, Wu, & Hellerstein, 1989; Wu, Kaempfer, Reid et al., 1989). These results initially suggested that isotope dilution of sulfamethoxazole-acetate may reflect PDH activation state. However, acetate is metabolized by many tissues in addition to the liver (Ballard, 1972). Therefore rate of appearance of sulfamethoxazole acetate may be underestimated using tracers of acetate if extrahepatic acetate metabolism is substantial. Furthermore, acetate label may exchange with oxaloacetate and then be recycled, via pyruvate kinase and PDH to acetyl CoA. Consequently, PDH activation could conceivably result in dilution of acetyl CoA with some labelled carbon, depressing the observed rate of appearance of acetyl CoA.

The 3H/14C ratio in acetaminophen-glucuronide may more accurately represent PDH activation state, i.e. activation of PDH is correlated with glycogen repletion (as indicated by an increase in the 3H/14C ratio during the transition from fasting to refeeding). If PDH were activated prematurely, glycogen deposition would be prevented. This would be indicated by a rapid increase in the 3H/14C ratio with refeeding or an elevated value during a fast. The

possible explanation for the increase in the ratio is that late in refeeding, as the rate of glycogenesis decreases, trioses returning to the liver from the periphery are redirected from glycogenesis, to hepatic lipogenesis (implying PDH activation). The implications of the foregoing work include the following. In rats, the close correlation between liver glycogen content and the ratio of 3H/14C in the excreted acetaminophen glucuronide suggests that the labeling pattern of UDP-glucose could serve as a non-invasive indicator of liver glycogen status in humans.

Based on the previous findings it may be inferred that in humans the occurrence of PDH activation late in the period of refeeding will normally be indicated by (Figures 8, 9 and 10):

1) increase proportion of direct pathway labeling of UDP-glucose (increased ratio of 1-d1 glucose to U-13C glucose in acetaminophen-glucuronide or increased ratio of UDP-glucose (acetaminophen-glucuronide) to plasma glucose enrichment),

2) increased rate of appearance of UDP-glucose early in refeeding, permitting glycogen accumulation (decreased enrichment from 1-d1 galactose in acetaminophen glucuronide), with plateauing of enrichment late in refeeding. During failure of mutual inhibition of glucose and fatty acid oxidation, such as may occur during HIV infection, one or more of these changes may not occur.

Figure 8

Hepatic Glucose/ Fatty Acid Metabolism During the Transition from Normal Starvation to Early Refeeding

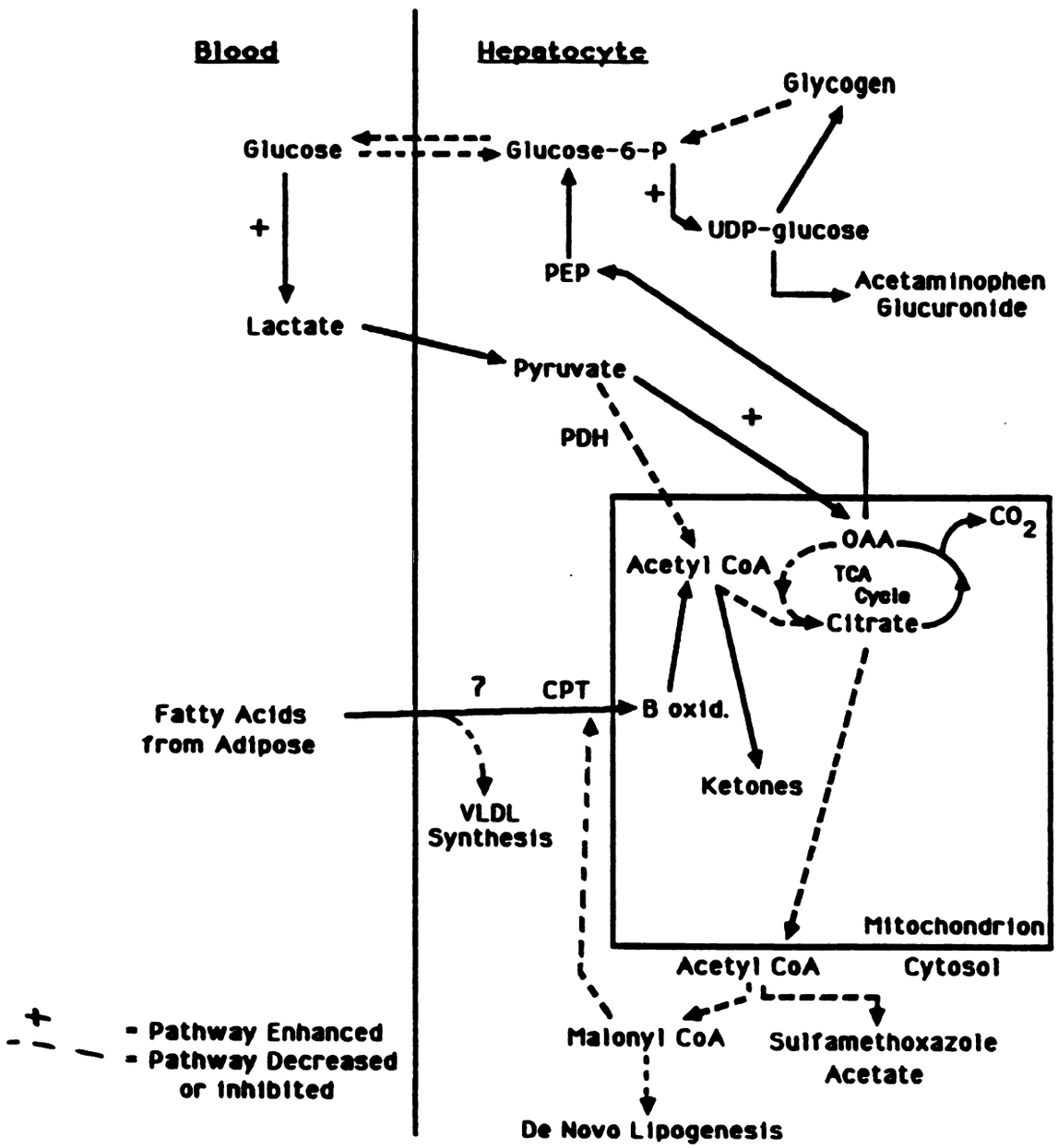


Figure 9

Hepatic Glucose/ Fatty Acid Metabolism During the Transition from Early Refeeding to Late Refeeding

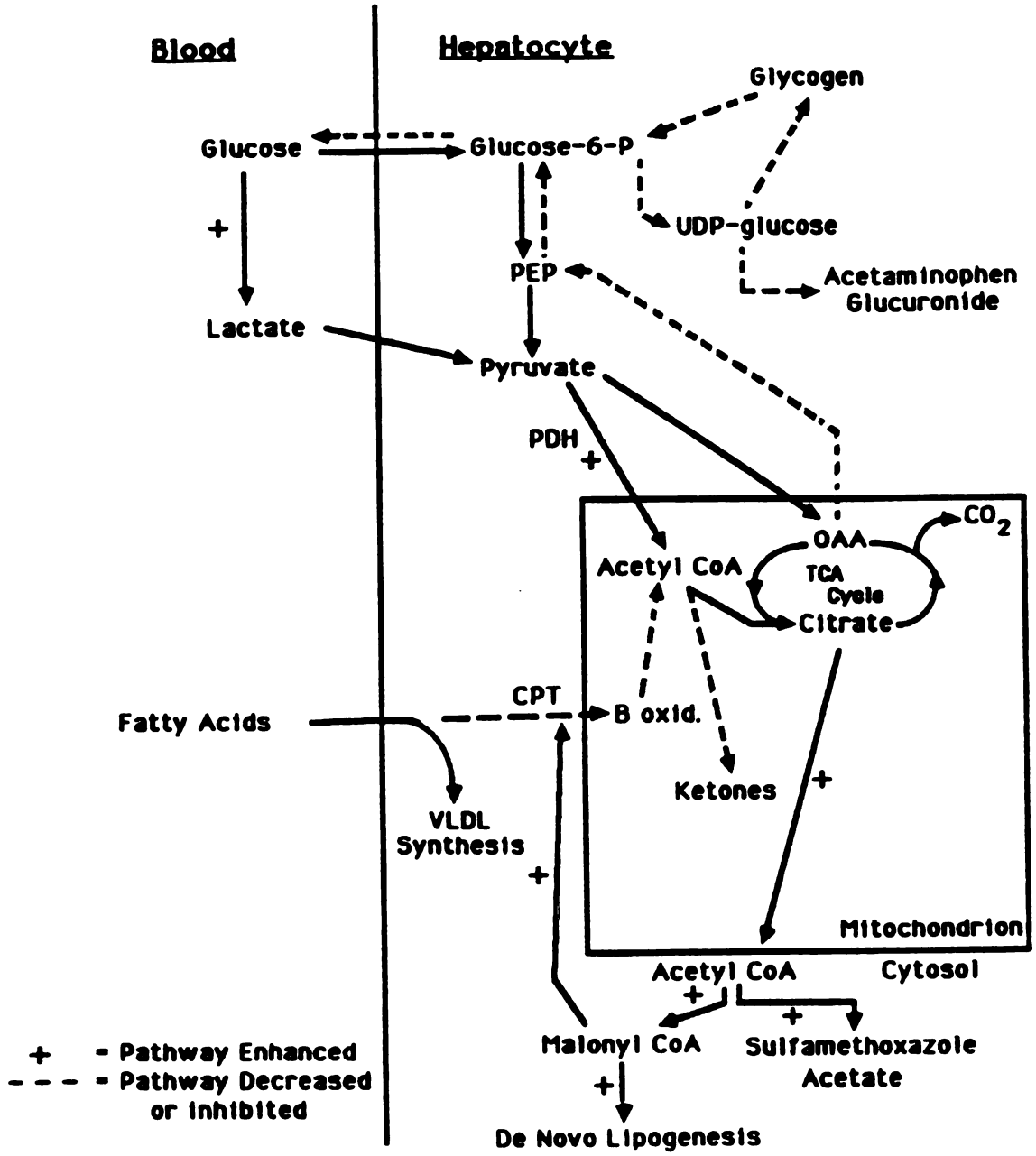
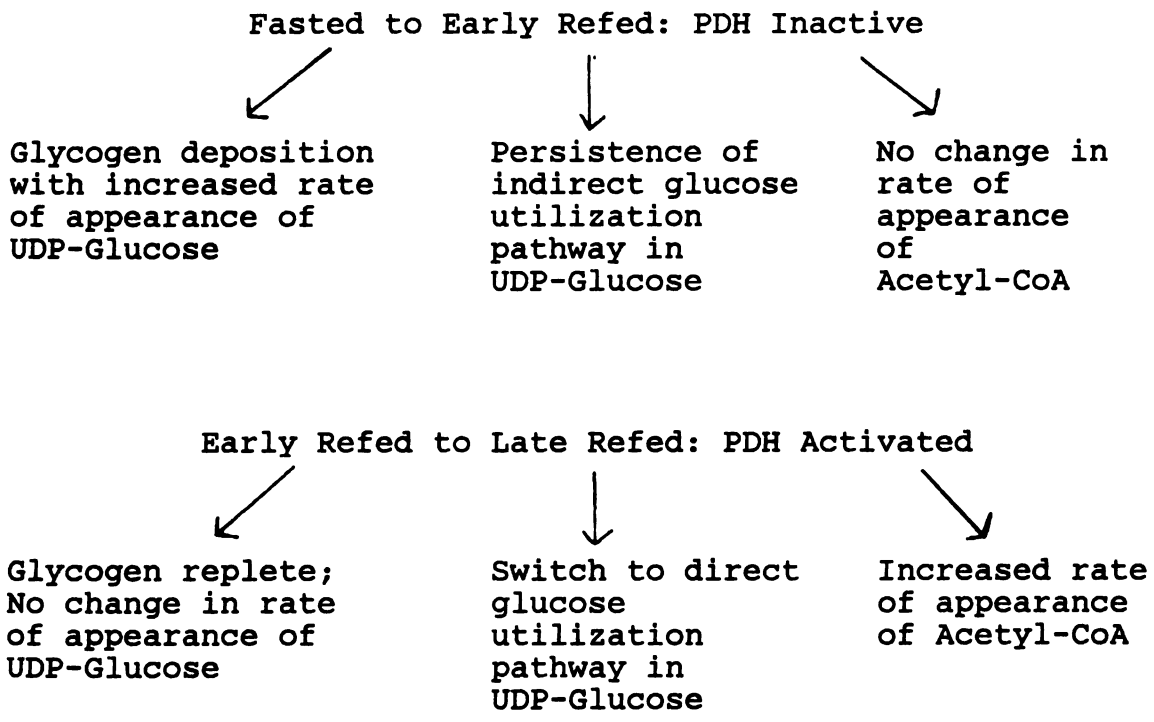


Figure 10

PDH Activation State as Inferred Using Non-Invasive Metabolic Probes



Application of the Acetyl and Glucuronide Probes to the Determination of Gluconeogenesis and UDP Gluconeogenesis

The true precursor for hepatic synthesis of plasma glucose and UDP glucose from trioses and TCA cycle intermediates is phosphoenolpyruvate (PEP)(See Figure 3). However it has been difficult to apply the precursor-product relationship and isotopic methods to the problem of determining rates of hepatic gluconeogenesis and UDP gluconeogenesis, due to the inability to sample hepatic PEP (Brosnan, 1982; Consoli, Kennedy, Miles et al., 1987; Consoli, Nurjhan, Capani et al., 1989; Hetenyi, 1982; Katz, 1985; Krebs, Hems, Weidemann et al., 1966; Strisower, Kohler, & Chaikoff, 1952; Weinman, Strisower, & Chaikoff, 1957). By using the acetyl probe to label the gluconeogenic pathway, the enrichment of PEP can be derived, permitting determination of gluconeogenesis and UDP gluconeogenesis, and thereby regulation of pathways of glucose carbon in the liver.

The rationale for this application of the acetyl probe is the occurrence of metabolic exchange of acetyl CoA carbon with oxaloacetate carbon in citrate as it traverses the TCA cycle. Oxaloacetate is at the intersection of gluconeogenesis and the TCA cycle in the liver. Acetate (acetyl CoA) label located in the one position (carboxyl label) becomes a carboxyl carbon of oxaloacetate, whereas acetate label in the two (methyl) position becomes a central

carbon of oxaloacetate. During sequential spins of the TCA cycle, the central carbons of oxaloacetate are decarboxylated more slowly than the carboxyl carbons. Depending on the relative rates of the TCA cycle decarboxylation (entry of acetyl CoA into citrate) and entry of TCA cycle intermediates (non-acetate flux), the central and carboxyl carbons of oxaloacetate will differentially exchange with non-acetate precursors and appear in plasma glucose and UDP glucose. This results in labeled acetyl CoA contributing labeled carbon (but not net carbon) to gluconeogenesis and UDP-gluconeogenesis. Thus relative incorporation of one versus two labeled acetate into plasma glucose and acetaminophen glucuronide represents acetate versus non-acetate carbon flux into oxaloacetate and subsequently into PEP.

Rates of hepatic gluconeogenesis and UDP gluconeogenesis can be calculated as follows (Katz, 1985; Weinman, Strisower, & Chaikoff, 1957):

1) determine X, which is the ratio of 2-13C acetate to 1-13C acetate label incorporation into plasma glucose and acetaminophen glucuronide (representing UDP glucose),

2) determine Y, which is the ratio of non-acetate to acetate flux, as X/(100-X),

3) determine the relative specific activity (RSA) of PEP, as (5 + 4y)/2(1 +y)(1 + 2y); this is the percent of PEP from acetyl CoA,

4) determine the absolute specific activity of PEP as RSA times specific activity of sulfamethoxazole acetate from methyl (2 position) labeled acetate,
5) determine the fractional synthesis of plasma glucose and UDP glucose from PEP as: specific activity of plasma glucose (or acetaminophen glucuronide)/ specific activity of PEP,
6) determine gluconeogenesis (plasma glucose) and UDP gluconeogenesis from rate of appearance of plasma glucose (or UDP glucose) times fractional synthesis from PEP of plasma glucose (or UDP glucose).

Using this method, in preliminary work it was possible to obtain values for hepatic gluconeogenesis rates in normal volunteers of 0.38 mg/kg/min during fasting and 0.57 mg/kg/min with refeeding (Hellerstein, Wu, Kaempfer et al. 1989). In these same subjects, UDP gluconeogenesis was 0.29 mg/kg/min during fasting and increased to .72 mg/kg/min with refeeding. These results represent an increase in partitioning of UDP gluconeogenic flux from 43% to 56% with refeeding, consistent with repletion of hepatic glycogen and reduced need for plasma glucose production.

Summary

The foregoing represents a conceptual framework for studying hepatic intermediary metabolism in vivo. The pathophysiology and clinical staging of HIV infection, hepatic function during HIV infection, and clinical considerations in the use of xenobiotic probes during HIV

infection are described below. Together, they provide an approach to determining possible abnormalities in regulation of the mutual inhibition of glucose and fatty acid metabolism in a human model of chronic infection, HIV infection.

Pathogenesis of HIV Infection

Infection with HIV (also called LAV, lymphadenopathy associated virus; HTLV-III, human T cell lymphotropic virus type III; or ARV, AIDS related virus) encompasses a wide spectrum of clinical presentations (Groopman, 1987a; Ho, Pomerantz, & Kaplan, 1987). These range from acute infection, to an asymptomatic state, to severe immunodeficiency with opportunistic infections and malignancies, and wasting (Yarchoan & Pluda, 1988). These presentations have in common, though, a common pathogenesis. HIV is trophic for CD4+ (T4 helper) lymphocytes (Fauci, 1988; Groopman, 1987a; Ho, Pomerantz, & Kaplan, 1987). Through poorly understood mechanisms, HIV enters CD4+ cells and integrates into the host DNA, where it may remain latent, (as in asymptomatic carriers of the virus), or may begin active replication in response to immunologic stimulation of the infected cell. Viral expression and dispersion results in death of the host cell, infection of other CD4+ cells (with their subsequent destruction), and ultimately depletion of CD4+ cells and a decrease in the ratio of CD4+ cells to CD8+ (cytotoxic suppressor) T-

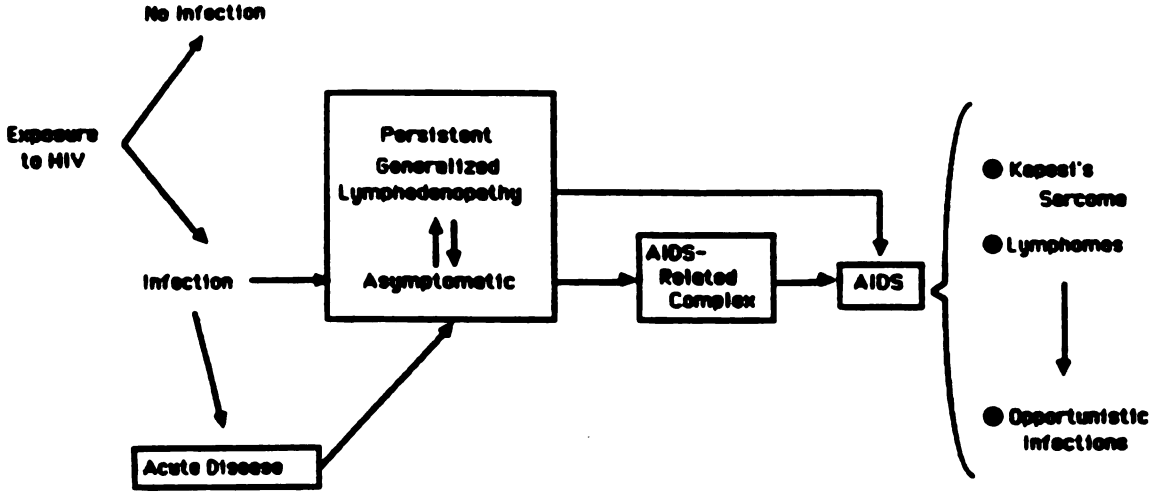
lymphocytes. Because HIV is incorporated into the host genes, HIV infection persists for the life of the individual (Ho, Pomerantz, & Kaplan, 1987).

The course of infection with HIV is depicted in figure 11. Exposure to HIV does not invariably result in infection, however factors which predict the outcome of HIV exposure have not been fully determined. The natural history of HIV infection is only beginning to be understood, because it has been difficult in most cases to determine the exact date of exposure (Volberding, 1986). The diversity of clinical outcomes of HIV exposure is reflected in figure 11.

The frequency and degree of immunologic abnormalities varies during the course of HIV infection. However, there seems to be a general trend whereby disease progression reflects deterioration in immune competence (Seligmann, Pinching, Rosen et al., 1987; Volberding, 1986). Individuals who develop superimposed opportunistic infections usually succumb within 2 years, whereas the long term outcome of asymptomatic HIV infection is unknown (Volberding, 1986). Duration of infection plays a major role in the progression to severe disease, through the effects of cofactors such as nutritional status, intercurrent sexually transmitted diseases, and genetic predisposition (Beach & Laura, 1983; Gray, 1983; Resler, 1988; Seligmann, Pinching, Rosen et al., 1987).

Figure 11

Course of Infection with Human Immunodeficiency Virus



Note. Reproduced, with permission, from: Seligmann, M., Pinching, A. J., Rosen, F. S. et al., "Immunology of human immunodeficiency virus infection and the acquired immunodeficiency syndrome." Annals of Internal Medicine. 1987; volume 107: p. 235.

Clinical Manifestations of HIV Infection

HIV infection can affect virtually every organ system, either directly or through immune suppression (Yarchoan & Pluda, 1988). Furthermore, many of the signs and symptoms of HIV infection are non-specific and may be indistinguishable from those due to secondary infections which are difficult to diagnose, in particular reactivation of cytomegalovirus (Groopman, 1986; 1987a).

Acute HIV infection following initial exposure resembles an episode of mononucleosis (Groopman, 1987b; Yarchoan & Pluda, 1988). Clinically, the individual presents with a sudden onset of non-specific symptoms, that may include: fever, headache, malaise, sore throat, arthralgias, myalgias, photophobia, lymphadenopathy (defined as lymph nodes greater than 1 cm. in diameter), and occasionally a transient erythematous rash. Acute HIV infection is not characterized by immunosuppression and it is frequently only diagnosed retrospectively by seroconversion (Groopman, 1987b). Persistent generalized lymphadenopathy (PGL) is thought to ensue from acute HIV infection, and denotes a relatively healthy individual demonstrating lymphadenopathy at two or more noninguinal sites over a period of three or more months. This progression, as well as that to more serious stages of HIV infection, however is associated with a progressive decline in CD4+ cells (Yarchoan & Pluda, 1988).

The clinical signs of ARC have not been uniformly defined, but can in general be described by the presence of generalized lymphadenopathy upon which are superimposed constitutional symptoms and a non-AIDS defining infection such as oral candidiasis or herpes zoster (Groopman, 1986; 1987b; Yarchoan & Pluda, 1988). The individual with ARC may present with intermittent or persistent diarrhea, leukopenia (usually lymphopenia), anemia, thrombocytopenia, fever, weight loss, night sweats, myalgia, fatigue, or malaise. Weight loss of the magnitude of 10-15% of previous body weight is an early manifestation of progression to AIDS (Groopman, 1986).

A diagnosis of AIDS is made in the presence of AIDS defining opportunistic infections (see Table 4). In addition, due to their prevalence and severity during HIV infection, diarrhea and malabsorption lasting for a month or longer, in conjunction with weight loss of 10% of preillness weight may now be considered an AIDS index diagnosis (Bartlett, Laughon, & Quinn, 1988).

The Centers for Disease Control classification scheme (table 4) represents HIV infection as a temporal hierarchy defined by groups of concurrent disease manifestations and constitutional symptoms (Centers for Disease Control, 1986). Within group IV, which contains the possible presentations of advanced HIV infection (including AIDS and ARC) a patient could occupy several subgroups.

Table 4

Summary of the Centers for Disease Control Classification System for HIV Infection

Group I — Acute infection

Group II — Asymptomatic HIV infection

Group III — Persistent generalized lymphadenopathy

Group IV — Other HIV disease

Subgroup A: Constitutional disease (Fever, diarrhea lasting < 1 month, 10% weight loss)

Subgroup B: Neurologic disease (dementia, myelopathy, or peripheral neuropathy)

Subgroup C: Secondary infectious diseases
Category C-1 AIDS defining infections (Pneumocystis carinii pneumonia, chronic cryptosporidiosis, toxoplasmosis, extraintestinal strogyloidiasis, isosporiasis, candidiasis (esophageal, bronchial, or pulmonary), histoplasmosis, mycobacterial infection with Mycobacterium avium complex of M. kansasii, cytomegalovirus infection, chronic mucocutaneous or disseminated herpes simplex virus infection, and progressive multifocal leukoencephalopathy.
Category C-2 Other infections

Subgroup D: Secondary cancers (Kaposi's Sarcoma, non-Hodgkin's lymphoma, primary brain lymphoma)

Subgroup E: Other conditions associated with HIV infection but not described by other Subgroups

Note. Reproduced, with permission, from: Centers for Disease Control, U. S. Department of Health and Human Services, "Classification system for human T-lymphotropic virus type III/ lymphadenopathy- associated virus infections." Annals of Internal Medicine. 1986; volume 105: p 235.

Complications of HIV Infection Affecting Hepatic Metabolism

Hepatic pathology during HIV infection is a consequence of three factors (Lebovics & Dworkin, 1987):

1) History of hepatitis virus exposure, which is common among groups at high risk of contracting HIV infection;

2) Infectious, neoplastic or treatment complications of the immunosuppressed state; and

3) Non-specific changes resulting from chronic debilitating illness, especially those related to malnutrition.

Clinical and histologic abnormalities of the liver have frequently been observed in patients with HIV infection. These have included serologic evidence of hepatitis B exposure, fatty degeneration (steatosis), hepatomegaly, elevation of serum transaminases and alkaline phosphatase, lymphocyte depletion, and granulomas due to secondary infections or adverse reactions to sulfonamides (Glasgow, Anders, Layfield et al., 1985; Lebovics, Thung, Schaffner et al., 1985; Nakanuma, Liew, Peters et al., 1986). No lesion attributable specifically to HIV infection itself, however, has been identified (Lebovics & Dworkin, 1987).

Opportunistic malignancies, especially Kaposi's Sarcoma (KS) (and to a lesser extent, diffuse undifferentiated B cell lymphoma), may complicate the picture of hepatic metabolism during HIV infection. Approximately 25% of reported cases of AIDS present with KS (Safai, 1987). Due to its association with impaired cellular immunity, the KS

associated with HIV infection (referred to as epidemic KS) is more aggressive than classical KS (Groopman, 1987a). Epidemic KS rarely manifests overt clinical symptoms early in its course, and frequently invades internal organs, including the liver (Gelmann & Broder, 1987; Groopman, 1986). In one series, hepatic involvement by KS was found in 14% (6 of 42) of cases at autopsy, none of which had been suspected prior to death (Glasgow, Anders, Layfield et al., 1985). Liver involvement tends to represent advanced KS (Groopman, 1987a). Moreover, epidemic KS combines the features of immune deficiency with malignancy, both of which can profoundly influence nutritional status and thereby hepatic function (Colman & Grossman, 1987).

Opportunistic infections can compromise hepatic function and have been identified in biopsy and autopsy specimens of hepatic tissue in HIV infected persons (Kotler, 1989; Kotler, Tierney, Altilio et al., 1989). Pathogenic organisms have included: Mycobacterium avium intracellulare, Mycobacterium tuberculosis, Histoplasmosis, Cryptococcosis, Cytomegalovirus, Herpes simplex virus, and Epstein-Barr virus (Lebovics & Dworkin, 1987). Hypoplasia of Kupffer cells, reflecting the effects of generalized infection has been observed in liver biopsy and necropsy specimens of patients with HIV infection (Nakanuma, Liew, Peters et al., 1986).

Weight loss, which may become severe and progressive

with wasting of body protein is a frequent concomitant of HIV infection and can contribute to deterioration of vital organ function including liver function (Garcia, Collins, & Mansell, 1987; Kotler, 1987; Kotler, Wang, & Pierson, 1985; O'Sullivan, Link, & Dalton, 1985). The factors which are known to contribute to HIV-associated malnutrition are summarized in Table 5. There are similarities between the clinical presentation of protein calorie malnutrition (PCM) and the malnutrition of HIV infection. However, HIV cachexia, unlike PCM, is refractory to aggressive nutritional intervention (Frei & Steigbigel, 1988; Gray, 1983; Groopman, 1986; Hopefl, 1988; Janson & Teasley, 1988).

Diarrhea and malabsorption are unusually prominent features of HIV infection and may play a major role in the pathogenesis of weight loss and wasting (Crocker, 1989). Although opportunistic pathogens may be identified at etiologic agents, lower gastrointestinal complications of HIV infection often consist of a non-specific enteropathy manifesting as persistent secretory diarrhea, not attributable to a specific organism (Bartlett, Laughon, & Quinn, 1988; Groopman, 1987b). This "AIDS enteropathy" has been associated with steatorrhea, abnormal D-xylose and 14C-glycerol-tripalmitin absorption, and histologic abnormalities of the small bowel and rectum (Dworkin, Wormser, Rosenthan et al., 1985; Gillin, Shike, Alcock et al., 1985; Kotler, Gaetz, Lange et al., 1984). While no

Table 5

Factors Contributing to Malnutrition During HIV Infection

Decreased Appetite and Decreased Nutrient Intake:

-Oral/esophageal mucositis and pain (lesions of opportunistic infections)
-Impaired motor ability
-Medication side effects
-Emotional distress
-Cognitive impairment (dementia)

Diarrhea and Malabsorption:

-AIDS enteropathy
-KS lesions
-Superimposed malnutrition
-Bowel pathogens

Hypermetabolism:

-Fever
-Concurrent infection

Metabolic Dysregulation:

-Cytokines

Alternative Therapies (e.g. macrobiotic diet)

precise cause of "AIDS enteropathy" has been identified, infection of enterocytes by HIV may be involved (Fox, Kotler, Tierney et al., 1989; Nelson, Reynolds-Kohler, Margaretten et al., 1988; Ullrich, Zeitz, Heise et al., 1989). In addition, there is recent evidence for the suppression of bowel lymphatic tissue function during HIV related diarrhea and malabsorption (Kotler, Scholes, & Tierney, 1987).

Use of Non-Invasive Xenobiotic Probes During HIV Infection

Sulfamethoxazole is employed in the treatment of at least three opportunistic infections associated with HIV infection (Kovacs & Masur, 1988; Shafer, Seitzman, & Tapper, 1989) and has been developed for use as a non-invasive probe of intracellular hepatic metabolism (specifically, cytosolic acetyl CoA) during HIV infection. Likewise, acetaminophen, a non-invasive probe of hepatic UDP-glucose, may be used to treat fever associated with HIV infection (Lebovics & Dworkin, 1987). While in the clinical treatment setting, adverse effects to both xenobiotics have been reported under certain circumstances in HIV infected individuals, these effects can be avoided during their use as non-invasive metabolic probes.

The major and minor adverse reactions to sulfonamides (administered as a combination of trimethoprim/ sulfamethoxazole) observed in one study are presented in Table 6. In this study, 41% of patients demonstrated major

Table 6

Major and Minor Adverse Reactions due to Prolonged Treatment at High Doses with Trimethoprim-Sulfamethoxazole during HIV Infection

Major adverse reactions:

- Neutropenia (<1000/uL)
- Thrombocytopenia (<50,000/uL)
- Increased liver enzymes* (>5 times upper limit of normal)
- Severe rash

Minor adverse reactions:

- Neutropenia (1000-1500/uL)
- Thrombocytopenia (50,000-100,000/uL)
- Anemia (hematocrit <30%)
- Azotemia (creatinine 1.5-3.0 mg/dL)
- Increased liver enzymes* (2-5 times upper limit of normal)
- Rash
- Nausea, vomiting
- Mental status changes
- Hyponatremia (<130 meq/dL)
- Hypocalcemia (<8.5 mg/dL)

*Alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase

Note. Reproduced, with permission, from: Wharton, J. M., Coleman, D. L., Wofsy, C. B. et al., "Trimethoprim-sulfamethoxazole or pentamidine for Pneumocystis carinii pneumonia in the Acquired Immunodeficiency Syndrome." Annals of Internal Medicine. 1986; volume 105: p. 41.

adverse reactions and 100% had minor adverse reactions but not until between 7 and 14 days after beginning treatment (Wharton, Coleman, Wofsy et al., 1986). In another study, an erythematous, maculopapular rash involving the whole body and accompanied by fever developed only after 8 to 12 days from initiation of sulfonamide treatment (adverse reactions within the first week were extremely rare); additional symptoms (table 6) were also reported and resolved upon discontinuation of the drug (Gordin, Simon, Wofsy et al., 1984). In this series, history of drug allergy did not predict adverse reaction to sulfonamide, nor did drug dosage or duration of drug treatment. In these studies, however, sulfamethoxazole was administered for a prolonged period and at high dosages: serum levels of sulfamethoxazole were maintained between 100-150 micrograms/ml and a median of 9.5 days of treatment were completed.

Comparison of sulfamethoxazole dosages employed during antiinfective therapy with dosages sufficient for use as a xenobiotic probe demonstrates that adverse effects may be avoided with this application of sulfamethoxazole in patients with HIV infection. During HIV infection complicated by pneumocystis pneumonia or salmonellosis, sulfamethoxazole is administered at a dosage of 75-100 mg./kg. every 6-8 hours for a period of up to 21 days. Prophylactic treatment may continue indefinitely at reduced dosages, if tolerated (Kovacs & Masur, 1988). Isospora

enteritis is treated with 3.2 gm. of sulfamethoxazole, four times a day. These dosages regimens contrast with that employed for sulfamethoxazole as a noninvasive probe of hepatic cytosolic acetyl CoA: 0.5 gm. (7-8 mg./kg.) every 4 hours for a total of four doses when used in an overnight study. This is one fourth to one fifth the therapeutic dose, given over a period of less than 24 hours (Hellerstein, 1988b). Thus, caution only appears to be warranted when sulfonamides are administered to HIV infected persons with a prior history of sulfonamide toxicity (Resnick & Herbst, 1987).

Adverse reactions to acetaminophen among HIV infected persons occur in conjunction with AZT (azidothymidine) treatment (Richman, Fischl, Grieco et al., 1987). AZT is a nucleoside analog with antiviral activity (Yarchoan & Broder, 1988). The half-life of AZT is approximately one hour and most administered AZT undergoes hepatic glucuronidation (to an inactive metabolite) followed by excretion in the urine (Laskin, de Miranda, & Blum, 1989). Concurrent administration of drugs which also undergo glucuronidation (such as acetaminophen), may therefore affect AZT metabolism and enhance its toxicity. (Toxicities observed during AZT treatment may include bone marrow suppression, headaches, nausea and vomiting, myalgias, confusion, and seizures.)(Yarchoan & Broder, 1988; Yarchoan,

Mitsuya, & Broder, 1989; Yarchoan, Mitsuya, Meyers, et al., 1989).

The dose of acetaminophen recommended for use as a non-invasive metabolic probe is 100 mg./hour for a period of 15 hours, which is in the lower range of the normal therapeutic dosage (Hellerstein, 1988b). In view of this low dosage and duration of administration, concurrent use of AZT appears to be the only contraindication to this application of acetaminophen during HIV infection.

Summary

To date there are no reports of the use of either isotopic methods or noninvasive probes of hepatic metabolism to study regulation of hepatic fat and carbohydrate metabolism during HIV infection. Much of the available evidence from the literature describing acute, self-limited, or chronic infection is, however, consistent with the model presented in Figure 1. The literature containing evidence for an abnormal metabolic response to starvation during general infection is described below.

Substrate Metabolism During Infection

Protein Metabolism During Infection

The striking feature of protein metabolism during infection is the coexistence of catabolic and anabolic processes. A dramatic increase in hepatic synthesis of acute phase reactant globulins accompanied by a depletion of circulating albumin are some of the primary laboratory

findings in both humans and animals during the acute phase response of infection (Kushner, 1982; Munro, 1974; Powanda, 1977; Williams, 1965; Woodward & Miraglia, 1964). (Acute phase globulins include ceruloplasmin, alpha-1-acid glycoprotein, fibrinogen, alpha-1-antitrypsin, haptoglobin, C-reactive protein, and serum amyloid-A-protein. Their precise functions are unknown but they are thought to mediate some of the host responses to tissue injury.) In addition, reductions in total circulating amino acids and increases in the ratio of phenylalanine to tyrosine have also been demonstrated in both animal and human models of experimental infection (Feigin & Dangerfield, 1967; Feigin, Klainer, Beisel et al., 1968; Powanda, Dinterman, Wannemacher et al., 1974; Wannemacher, Powanda, Pekarek et al., 1971; Wannemacher, Pekarek, Bartelloni et al., 1972; Wannemacher, Klainer, Dinterman et al., 1976; Wannemacher, 1977). These changes could not be explained by either reduced intake (Wannemacher, Pekarek, Bartelloni et al., 1972) or enhanced urinary excretion (Wannemacher, Dinterman, Pekarek et al., 1975) of amino acids, although isolated skeletal muscle from infected rats was found to release more phenylalanine and less tyrosine than controls (Wannemacher, Klainer, Dinterman et al., 1976). (Phenylalanine levels are a qualitative indicator of the balance between muscle protein synthesis and degradation because phenylalanine is not metabolized by muscle. Thus increases in phenylalanine

reflect net protein catabolism (Vary, Siegel, Zechnich et al., 1988).

Negative nitrogen balance (implying protein malnutrition) with wasting of body tissues is one of the cardinal clinical features of infection (Munro, 1974). Using metabolic balance techniques, negative nitrogen balance has been observed during experimental viral and bacterial infection in man (Beisel, 1966; Beisel, Sawyer, Ryll et al., 1967). Compared with pair-fed controls, infected subjects continued to have excessive urinary nitrogen loss in the presence of reduced food intake, indicating breakdown of body protein. This implies a failure of the normal compensatory mechanism of conserving body protein during caloric deficit. That the site of protein breakdown was skeletal muscle was supported by the observation of increased urinary excretion of 3-methylhistidine and creatinine (metabolic products of contractile protein catabolism) during experimental infection in humans (Wannemacher, Dinterman, Pekarek et al., 1975) and rats (Powanda, Wannemacher, & Cockerell, 1972).

Tracers of individual amino acids (e.g. phenylalanine, leucine and cycloleucine) have been employed to further document the redistribution of amino acids from muscle to liver during infection in animal models (See Table 7). (Leucine and phenylalanine are essential amino acids and cycloleucine is a non-metabolizable analog of leucine whose

Table 7
Summary of Studies Describing Effects of Infection on Hepatic Protein Metabolism

Investigators	Species	Model of Infection	Major Findings
Lust, 1966	mouse	D. pneumoniae Venezuelan equine encephalitis	Increased in vivo incorporation of U-14C leucine into hepatic microsomal protein (n.r.); decreased incorporation into muscle microsomal protein (n.r.)
Powanda, Wannemacher, & Cockerell, 1972	rat	D. pneumoniae (protein deficient diet)	Increased incorporation of 3H-leucine into liver and serum protein ($p<0.005$) Increased concentration and 3H-leucine labeling of acute phase globulins; decreased concentration and labeling of plasma albumin ($p<0.005$) Decreased 3H-leucine incorporation into muscle protein ($p<0.001$)
Powanda, Dinterman, Wannemacher et al., 1974	rat	F. tularensis (fasted)	Increased 14C-cycloleucine incorporation into liver ($p<0.001$) Decreased incorporation into muscle ($p<0.05$)
Rappaport, Lust, & Beisel, 1968	mouse	D. pneumoniae	Increased incorporation of U-14C leucine into hepatic microsomal protein ($p<0.05$); decreased incorporation into muscle protein (n.r.)
Vary & Murphy, 1989; Vary, Siegel, & Zechnech et al., 1988	rat	B. fragilis E. coli	Increased plasma and skeletal muscle lactate concentrations ($p<0.05$)

Table 7
Summary of Studies Describing Effects of Infection on Hepatic Protein Metabolism (continued)

Investigators	Species	Model of Infection	Major Findings
Vary, Siegel, Nakatami, Sato, & Aoyama, 1986b	rat	B.fragilis E. coli	Decreased skeletal muscle PDH activity ($p<0.005$)
Vary, Siegel, Tall, et al., 1988	rat	B. fragilis E. Coli	Decreased incorporation of 3H-phenylalanine into skeletal muscle protein ($p<0.005$) Reduced skeletal muscle protein content ($p<0.02$)
Wannemacher, Powanda, Pekarek, et al., 1971	rat	D. pneumoniae	Increased incorporation of 3H-leucine into plasma protein; decreased incorporation into muscle protein ($p<0.01$)
Wannemacher, Klainer, Dinterman et al., 1976	rat	S. pneumoniae	Increased incorporation of 14C-phenylalanine into serum protein ($p<0.01$); decreased incorporation into skeletal muscle ($p<0.01$)
Wannemacher, Neufeld, & Canonico, 1976	rat	Pneumococcus	Increased 14C-alanine incorporation into 14C-glucose (n.r.) Increased glucose turnover (n.r.)

Note: B. fragilis (Bacteroides fragilis); D. pneumoniae (Diplococcus pneumoniae); E. coli (Escherichia coli); F. tularensis (Franciscella tularensis); S. pneumoniae (Streptococcus pneumonia); n.r. (statistical significance not reported)

uptake has been used to indicate amino acid uptake by body tissues.) Increased incorporation of radio-labelled leucine, cycloleucine, and phenylalanine into hepatic microsomal protein and acute phase reactant globulins have been demonstrated in different species using a variety of infectious agents. These changes occurred in conjunction with decreased incorporation of label into muscle protein (Lust, 1966; Powanda, Wannemacher, & Cockerell, 1972; Powanda, Dinterman, Wannemacher et al., 1974; Rapoport, Lust, & Beisel, 1968; Vary, Siegel, Tall et al., 1988; Wannemacher, Powanda, Pekarek et al., 1971; Wannemacher, Klainer, Dinterman et al., 1976; Williams, 1965).

Amino acids, especially, alanine, and lactate are precursors for hepatic gluconeogenesis (glucose synthesis). Increased hepatic alanine concentration was observed in fasted infected rats (Wannemacher, Powanda, Pekarek et al., 1971). In addition, 14C alanine incorporation into 14C glucose was enhanced, in conjunction with higher glucose turnover (replacement by liver glucose production) <u>in vivo</u> (Wannemacher, Klainer, Dinterman et al., 1976). Lactate release in peripheral tissues was elevated in an ad lib fed rat model of acute infection (Vary & Murphy, 1989). This occurred in conjunction with decreased PDH activity in skeletal muscle (Vary, Siegel, Nakatani, Sato, & Aoyama, 1986b).

The above studies support the hypothesis that a

regulatory disturbance involving increased net protein catabolism of skeletal muscle occurs during infection. The branch chain amino acids (leucine, isoleucine, and valine, which can only be oxidized by skeletal muscle) are oxidized or converted to alanine or glutamine to provide gluconeogenic substrates (Nawabi, Block, Chakrabarti et al., 1990). Phenylalanine released during skeletal muscle proteolysis can not be metabolized by skeletal muscle and therefore achieves higher blood concentrations relative to other amino acids (Wannemacher, 1977). The mobilized phenylalanine can be used for hepatic protein synthesis. In addition, regulation of skeletal muscle PDH may be altered during infection and contribute to accelerated gluconeogenesis. Acute phase globulins, enzymes for hepatic metabolism, and glucose are thus produced at the expense of decreased synthesis of muscle protein and circulating albumin.

Glucose Metabolism During Infection

Hypoglycemia, hepatic glycogen depletion, and impaired hepatic gluconeogenesis occur in animal models of lethal, fulminating infection and endotoxemia (Berry, Smythe, & Young, 1959; LaNoue, Mason, & Daniels, 1968; Moore, Johnson, & Berry, 1977; Snyder, Deters, & Ingle, 1971; Wannemacher, Neufeld, & Canonico, 1976). In contrast, the picture of glucose metabolism during acute, but not life-threatening infectious illness is quite different. In humans, impaired

glucose clearance and exaggerated insulin and glucagon response during glucose loading (glucose tolerance test) have been documented in experimental infections (Rayfield, Curnow, George et al., 1973; Shambaugh & Beisel, 1967) and during endotoxin administration (Rayfield, Curnow, Reinhard et al., 1977), implying relative insulin unresponsiveness (Vary & Murphy, 1989).

The ratio of circulating insulin to glucagon, and not their respective concentrations, determine the metabolic effects of insulin and glucagon (Parilla, Goodman, & Toews, 1974). Moreover, the insulin:glucagon ratio is inversely related to the need for gluconeogenesis, i.e. the ratio is low during normal starvation (Unger, 1971). During experimental infection in man (Rocha, Santeusanio, Faloona et al., 1973) and animals (George, Rayfield, & Wannemacher, 1974; Zenser, DeRubertis, George et al., 1974), fasting hyperglucagonemia and depression of the insulin: glucagon ratio have been demonstrated, supporting a role for glucagon in promoting increased gluconeogenesis during inflammatory stress. Basal hepatic cyclic AMP levels were elevated in infected rats (Curnow, Rayfield, George et al., 1976; Zenser, DeRubertis, George et al., 1974). Hepatic cyclic AMP levels (mediating hepatic glucagon action), however were blunted during glucagon infusion in comparison to controls in infected rats, suggesting hepatic insensitivity to glucagon.

Several abnormalities of hepatic glycogen metabolism have been observed during experimental infection in animals (See Table 8). Fasted rats were found to deplete glycogen more rapidly than controls, with reduced hepatic glycogen synthase activity (during fasting and glucose loading) and increased glycogen phosphorylase activity (during fasting)(Curnow, Rayfield, George et al., 1976). Fasted guinea pigs repleted glycogen more slowly in response to exogenous glucose (Singh, Venkitasubramanian, & Viswanathan, 1963), but these changes were correlated with decreased activities of hepatic glycogen synthase, glycogen phosphorylase, and phosphoglucomutase and increased hepatic glucose-6-phosphatase activity (Singh, Bhargava, Venkitasubramanian et al., 1963). Sequential changes in liver glycogen during infection in guinea pigs were manifested by a period of glycogen depletion during early infection, followed by glycogen repletion later in the infectious process (Paretsky, Downs, & Salmon, 1964). Glycogen synthase and phosphorylase were activated and inactivated appropriately.

Although the mechanisms underlying the apparent inability to maintain glycogen stores were not explained, the alterations in glucose metabolism taken as a whole seem to indicate the persistence of a glucose-based fuel economy during infection. This occurs in an endocrine environment favoring catabolism of body protein and inability to

Table 8
Summary of Studies Describing Hepatic Glycogen Metabolism During Infection

Investigators	Species	Model of Infection	Major Findings
Curnow, Rayfield, George et al., 1976	rat	D. pneumoniae (fasted)	More rapid hepatic glycogen depletion, reduced glycogen synthase activity, increased glycogen phosphorylase activity ($p<0.05$) Impaired glycogen synthase activation in response to exogenous glucose ($p<0.05$)
Paretsky, Downs, & Salmon, 1964	guinea pig	C. burnetti	Glycogen depletion and decreased glycogen synthase activity by day 3-4 after inoculation; glycogen repletion and increased phosphorylase activity by day 11 (n.r.)
Singh, Venkitasubra-manian, & Viswanathan, 1963	guinea pig	M. tuberculosis (fasted)	Decreased synthesis of hepatic glycogen from exogenous glucose ($p<0.001$)
Singh, Bhargava, Venkitasubra-manian et al., 1963	guinea pig	M. tuberculosis (fasted)	Decreased activity of hepatic glycogen synthase, phosphorylase, and phosphoglucomutase ($p<0.001$) Increased hepatic glucose-6-phosphatase activity ($p<0.05$)

Note: C. burnetti (Coxiella burnetti); D. pneumoniae (Diplococcus pneumoniae); M. tuberculosis (Mycobacterium tuberculosis); n.r. (statistical significance not reported)

preserve glucose precursors and glycogen.

Lipid Metabolism During Infection

Alterations in circulating lipid moieties have been frequently observed during infectious illness (Beisel & Fiser, 1970), consisting primarily, but not consistently of hypertriglyceridemia in models of experimental infection in animals (Farshtchi & Lewis, 1968; Fiser, Denniston, & Beisel, 1972; Gallin, O'Leary, & Kaye, 1970; Hirsch, McKay, Travers et al., 1964; Kaufmann, Matson, Rowberg et al., 1976; and many others) and in man (Gallin, Kaye, & O'Leary, 1969; Lees, Fiser, Beisel et al., 1972). In addition lipoprotein patterns have been described in humans diagnosed with a variety of infectious illnesses (Akerlund, Carlson, & Jarstrand, 1986; Kerttula & Weber, 1986; Sammalkorpi, Valtonen, Kertulla et al., 1988; and many others).

Lipid profiles, per se, however have been uninformative regarding lipid metabolism during infection since they provide no information on regulation of intracellular or interorgan fuel flow. For example, reduced plasma lipolytic activity following heparin injection, indicating reduced lipoprotein lipase (LPL) and hepatic lipase (HL) activity has been observed in infected rhesus monkeys (Kaufmann, Matson, Rowberg et al., 1976) and in humans with naturally occurring bacterial and viral infections (Sammalkorpi, Valtonen, Kertulla et al., 1988). LPL and HL hydrolyze triglyceride for uptake by peripheral and hepatic tissues.

Decreased post-heparin lipolytic activity is therefore consistent with previously described elevations in plasma triglyceride and the proposal of decreased utilization of lipoprotein triglyceride by peripheral tissues. This finding, however, fails to implicate the potential role of altered hepatic metabolism in triglyceride synthesis, transport and utilization during infection.

The normal adaptation to starvation includes mobilization of fatty acids by adipose tissue and their subsequent oxidation to ketones by the liver. In the liver fatty acids are partitioned into either the mitochondria for beta oxidation and ketogenesis or into the cytosol for re-esterification with glycerol to form triglyceride and lipoproteins. Ketogenesis depends on the transport of fatty acids into the mitochondria by a carrier enzyme, carnitine palmitoyl transferase (CPT), which is regulated by a cytosolic metabolic intermediate involved in fatty acid synthesis, malonyl CoA. Re-esterification depends on a supply of glucose or glycolytic intermediates for glycerol phosphate synthesis. Both processes depend on the nutritional and endocrine environment.

When the liver is provided with glucose or glucose precursors, acetyl CoA produced in the mitochondria from pyruvate is transported to the cytosol in the form of citrate (through condensation with oxaloacetate). Cytosolic acetyl CoA is converted to malonyl CoA by the enzyme acetyl

CoA carboxylase. Acetyl CoA carboxylase is activated in the presence of insulin and citrate (implying availability of oxaloacetate). Under these conditions malonyl CoA inhibits CPT and therefore ketogenesis.

With fasting, glucose is not available for either glycerol phosphate or malonyl CoA synthesis by the liver, hepatic oxaloacetate (and therefore citrate) is not being replenished, and insulin levels are low. CPT is not inhibited and fatty acids enter the mitochondria. Beta oxidation yields acetyl CoA which is directed into ketogenesis.

Therefore, during feeding glucose will be used for glycerol and fatty acid synthesis, fatty acids synthesized de novo will be esterified to triglyceride, and fatty acid mitochondrial transport and oxidation will be inhibited. During a fast (glucose not available), fatty acid oxidation (mobilized from adipose tissue) will predominate. This regulatory mechanism integrates hepatic glucose oxidation, fatty acid oxidation, and fatty acid synthesis.

A number of studies support the hypothesis that during infection, fasting results in partitioning of fatty acids into lipogenesis rather than into ketogenesis, implying a futile cycle of adipose lipolysis and hepatic re-esterification (See Table 9). Depressed levels of circulating ketones and fatty acids have been demonstrated in animal models of bacterial and viral infection, endotoxin

Table 9
Summary of Studies Describing Hepatic Lipid Metabolism During Infection

Investigators	Species	Model of Infection	Major Findings
Canonico, Ayala, Rill et al., 1977	rat	S. pneumoniae (fasted)	Increased conversion of 1-14C acetate to all lipid fractions by isolated hepatocytes ($p<0.05$)
Kaminski, Neufeld, & Pace, 1979	rat	S. pneumoniae Endotoxin (E. coli)(fasted)	Decreased serum ketone and FFA's in all groups (n.r.) Increased peripheral and portal insulin and glucagon ($p<0.001$) in S. pneumoniae
Neufeld, Pace, Kaminski et al., 1980	rat	S. pneumoniae F. tularensis Venezuelan equine encephalitis Endotoxin (E. Coli)(fasted)	Depressed plasma ketones ($p<0.001$) and FFA (n.r.) Increased plasma insulin ($p<0.001$)
Neufeld, Pace, & White, 1976	rat	S. pneumoniae F. tularensis S. typhimurium (fasted)	Decreased hepatic ketones (3-beta-hydroxybutyrate)($p<0.001$)
Neufeld, Kaminski, & Wannemacher, 1977 Pace, Beall, Neufeld et al., 1977	rat	S. pneumoniae (fasted)	Decreased liver CPT (n.r.) Palmitoyl carnitine oxidized normally by isolated mitochondria (n.r.)

Table 9
Summary of Studies Describing Hepatic Lipid Metabolism During Infection (continued)

Investigators	Species	Model of Infection	Major Findings
Pace, Beall, Foulke et al., 1978; Wannemacher, Pace, Beall et al., 1979	rat	S. pneumoniae F. tularensis (fasting)	Decreased ketone production from oleic acid in isolated perfused livers ($p<0.01$) Increased incorporation of 1-14C oleic acid into liver lipids; decreased incorporation into ketones in isolated perfused livers ($p<0.01$)
Vary, Siegel, Nakatani, Sato, & Aoyama, 1986a	rat	B. fragilis E. coli (fed)	Increased hepatic malonyl CoA levels ($p<0.05$)

Note: B. fragilis (Bacteroides fragilis); CPT (carnitine palmitoyl transferase); E. coli (Escherichia coli); FFA (free fatty acids); F. Tularensis (Franciscella tularensis); S. pneumoniae (Streptococcus pneumoniae); S. typhimurium (Salmonella typhimurium); n.r. (statistical significance not reported)

inflammation, and turpentine inflammation during fasting (Neufeld, Pace, & White, 1976; Neufeld, Pace, Kaminski et al., 1980; Kaminski, Neufeld, & Pace, 1979). These changes were associated with increased peripheral and portal levels of insulin and glucagon, and did not occur in untreated diabetic rats, suggesting a potential endocrine mechanism for the altered fatty acid availability and ketogenesis.

CPT transports long chain fatty acids across the mitochondrial membrane. Livers of fasted infected rats were found to have reduced levels of CPT, while isolated hepatic mitochondria were able to oxidize CPT-bound fatty acids normally. These changes were correlated with plasma ketone levels (Pace, Beall, Neufeld et al., 1977; Neufeld, Kaminski, & Wannemacher, 1977). Using fasted, experimentally-infected rats, isolated perfused livers produced less ketones from oleic acid (a long chain fatty acid) and isolated mitochondria showed normal oxygen consumption (Wannemacher, Pace, Beall et al., 1979; Pace, Beall, Foulke et al., 1978). Liver content of ketones was depressed in fasted infected rats (Neufeld, Pace, & White, 1976). In ad lib fed, infected rats, depressed hepatic ketone levels were accompanied by increased hepatic malonyl CoA content (Vary, Siegel, Nakatani, Sato, & Aoyama, 1986a). This series of observations suggested that during infection a defect in mitochondrial transport of long chain fatty acids, involving inhibition of CPT by malonyl CoA, could be

occurring, thus impairing ketogenesis.

Enhanced hepatic lipogenesis during infection was initially suggested by the observation of increased liver weight and total lipid and triglyceride content in animal models (Hirsch, McKay, Travers et al., 1964; Paretsky, Downs, & Salmon, 1964; Wannemacher, Pace, Beall et al., 1979). That acetyl CoA was being partitioned into lipogenic (de novo fatty acid synthesis) rather than ketogenic pathways was supported by the increased conversion of 1-14C acetate to all lipid fractions by isolated hepatocytes from fasted, infected rats (Canonico, Ayala, Rill et al., 1977). Furthermore, 1-14C oleic acid, when infused into isolated perfused livers of fasted, infected rats, were incorporated preferentially into lipids rather than ketones (Wannemacher, Pace, Beall et al., 1979).

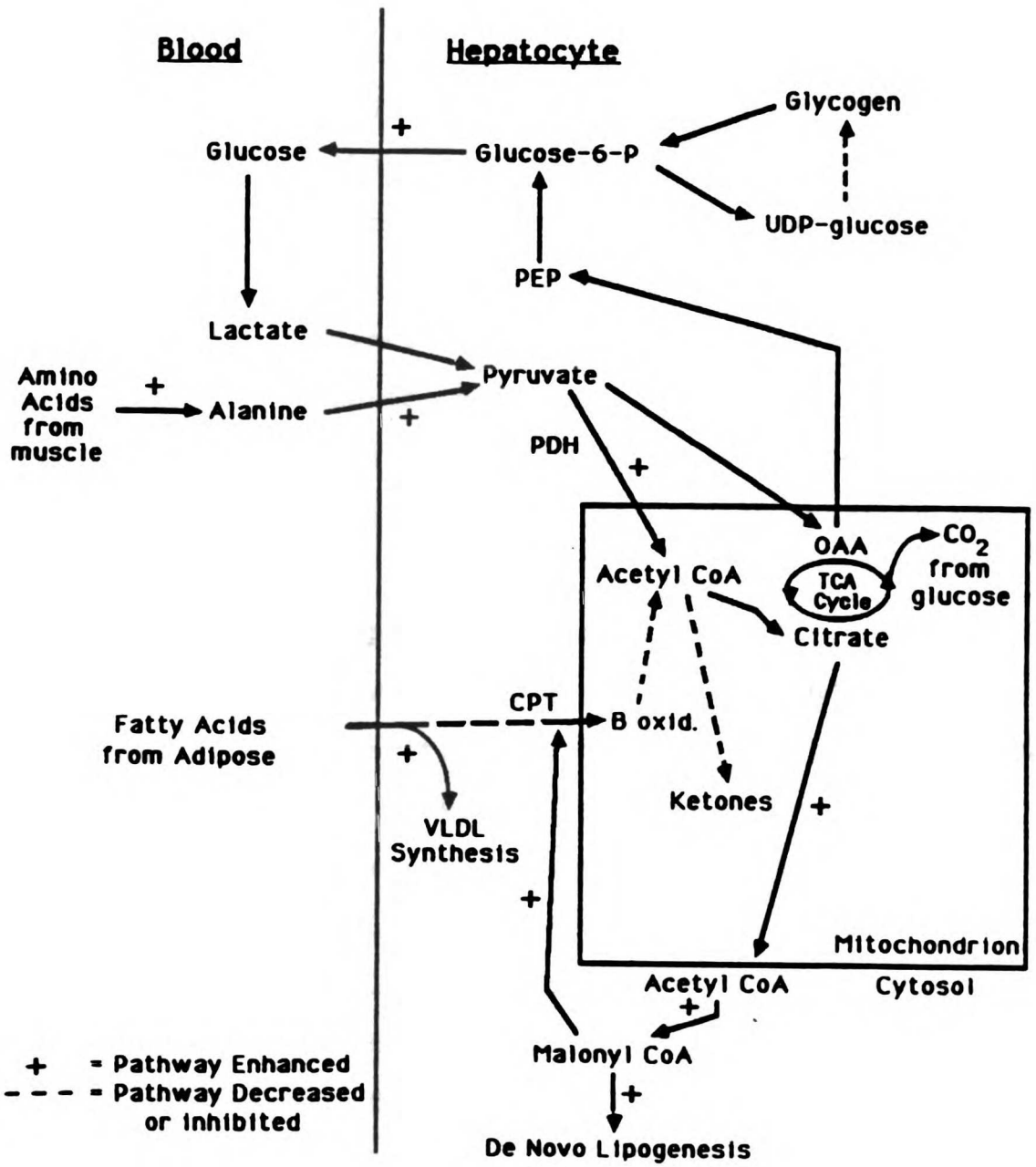
There exists, therefore, some evidence, primarily from studies of intracellular metabolites, for abnormal hepatic regulation of lipid metabolism during infection. Figure 12 summarizes the abnormalities in hepatic substrate metabolism which may be inferred from the foregoing review. The intracellular pathways whereby fatty acid and ketone metabolism are regulated in relation to glucose production and oxidation, and protein synthesis and catabolism during an infectious process remain to be determined.

Summary

The dramatic wasting of body tissue, one of the most

Figure 12

Failure of Metabolic Adaptation to Starvation during Infection



overt manifestations of infectious illness, was addressed by numerous studies during the 1960's and 1970's. This now classic body of literature, surveyed in the present review, fostered not only the discovery of cytokines but also the recent advances in the understanding of nutritional effects of trauma and sepsis.

From the foregoing review it is clear that the characterization of the metabolic response to starvation during infection is far from complete. Studies of substrate metabolism in the infected host have thus far focused primarily on intermediate starvation. The major sources of this information, however, have been levels of circulating metabolites; and only in animal studies have direct measurement of intracellular metabolites been possible to date.

The metabolic response to starvation involves the regulation of fuel flow between liver, muscle and adipose tissues during the transitions from fasting to refeeding and the converse. Methods to study disorders of this dynamic, multiorgan process, therefore, must be able to separate the roles of the individual organs (liver versus muscle versus adipose) and also enable access to levels of intracellular metabolites during ongoing metabolic changes continuously <u>in vivo</u>. Determination of the site of intracellular metabolic dysregulation during the fasting to feeding transition in a human model of infection (e.g. HIV infection) is now

accessible using non-invasive probes and is the focus of the present study.

Chapter III

Methodology

The purpose of this study was to describe regulation of hepatic intermediary metabolism during HIV infection in a sample of individuals representing a range of clinical presentations. Pathway of hepatic glucose utilization and fractional synthesis of plasma glucose (hepatic gluconeogenesis)(GNG) and UDP-glucose (UDP-gluconeogenesis)(UDP-GNG) from PEP were selected as indicators of the metabolism of glucose and glucose precursors by the liver. Pathway of glucose utilization is measured using the glucuronide probe; hepatic GNG and UDP-GNG are measured using both the glucuronide and acetyl probes. This chapter describes the methodology employed in addressing the research questions, beginning with the operational definitions. These are followed by the research design, sample selection criteria, instrumentation and procedures, limitations of the study, and method of data analysis.

Operational Definitions

AIDS: Stage IVc to IVe in the Centers for Disease Control classification scheme for HIV infection (Centers for Disease Control, 1986); also HIV infection associated with more than 10% involuntary loss of preillness body weight in conjunction with diarrhea and malabsorption lasting for a month or longer.

Clinically stable HIV infection: Stage II to IVa in the Centers for Disease Control classification scheme for HIV infection (Centers for Disease Control, 1986)

Fasting: caloric deprivation during the overnight period lasting from 18:00 to 09:00 (total of 15.0 hours), representing the period of early starvation, also called the "post-absorptive" state

Fractional gluconeogenesis: fraction of plasma glucose synthesized from hepatic phosphoenolpyruvate (e.g. from hepatic gluconeogenesis) determined according to the method of Weinman, Strisower, & Chaikoff (1957) as elaborated by Katz (1985) using sequential infusions of 1-13C acetate and 2-13C acetate

Fractional UDP gluconeogenesis: fraction of hepatic UDP glucose synthesized from hepatic phosphoenolpyruvate (e.g. from hepatic gluconeogenesis) determined by the method of Katz (1985) and Weinman, Strisower, & Chaikoff (1957) using sequential infusions of 1-13C acetate and 2-13C acetate

Hepatic glucose utilization pathway: the route by which glucose carbon enters hepatic intermediary metabolic pathways. The two routes of interest are 1) direct hepatic uptake of glucose, and 2) glycolytic metabolism of glucose to trioses, followed by hepatic gluconeogenic uptake of those trioses. The direct pathway fractional contribution to hepatic UDP-glucose

synthesis (that is, the fraction of hepatic UDP-glucose that comes from direct uptake of the intact glucose molecule) is experimentally determined by the ratio of the enrichment of acetaminophen- glucuronide relative to plasma glucose during constant infusion of 1-d1 glucose

Steady state: serial measurements attaining constant values (plus or minus 10%) after an overnight fast or more than 3 hours of refeeding

Refeeding: intravenous infusion of glucose (7 mg/kg/hr) or hourly consumption of ensure (3.1 ml/kg/hour), following fasting to produce a steady state rate of caloric repletion

Usual body weight: stable weight maintained during the previous 12 months. Usual body weight will be obtained from medical records.

Weight loss: involuntary loss of usual body weight during the 6 month period preceding the study: 5-10% for clinically stable HIV infection, (>10% defining AIDS).

Research Design

The study objectives were addressed using an exploratory descriptive design. Pathways of hepatic glucose utilization during fasting and subsequent refeeding were described in three groups of subjects representing a range of HIV infection and weight loss. In part of the sample fractional gluconeogenesis and fractional UDP-

gluconeogenesis during fasting were described.

Sample

Three groups of subjects, representing a range of HIV infection as compared with healthy controls were studied: 1) HIV negative normal volunteers, 2) clinically stable HIV infected subjects with no weight loss or complications, and 3) HIV infected subjects with weight loss and complications (AIDS). Subjects were recruited by advertisement, and by referral by members of the AIDS Nutrition Network, an organization of health professionals that provides nutritional counseling to HIV infected individuals. A sample size of 5-10 per group was considered to be the minimum needed to provide an indication of the range of values within groups for study parameters in a descriptive study.

Only males between the ages of 20 and 55 were eligible to participate in the study. A detailed medical history and physical examination was performed to exclude individuals whose metabolism might be altered by coexisting conditions, other than the sequelae of HIV infection, (specifically, liver disease, kidney disease, diabetes mellitus, or intercurrent infection). Potential subjects were also excluded if they had a history of alcohol or intravenous drug abuse, or were currently using steroid medications or other drugs that affect substrate metabolism. Alcohol abuse was defined as a level of intake that interferes with the

ability to perform usual daily functions. HIV infected subjects taking AZT were not excluded but the medication was withheld following the 6 p.m. dose the evening prior to each infusion until the completion of the infusion.

In addition, each group adhered to the following criteria:

I. Normal volunteers

a. HIV negative (documented serologically)

b. 100% (or greater) of usual body weight

II. Clinically stable HIV infection without weight loss

a. Stage II-IVa HIV infection (Centers for Disease Control classification system)(Centers for Disease Control, 1986)

b. More than 95% of usual body weight

III AIDS

a. Stage IVc-IVe HIV infection (Centers for Disease Control classification system) (Centers for Disease Control, 1986) or diarrhea of a month or more duration in conjunction with weight loss

b. More than 10% involuntary loss of usual body weight during the previous 6 months (documented)

Approval for the author's participation in study procedures was obtained from the Committee on Human Research at UCSF (approval # H3049-03703-01). Certification was also obtained from the National Cancer Institute (a stipulation of predoctoral training grant #1F31 CA 08000-05).

Instrumentation

Pathway of hepatic glucose utilization during fasting and refeeding was determined using the glucuronide probe. Acetaminophen and 1-d1 glucose were infused during an overnight (30 hour) admission; metabolites (acetaminophen glucuronide and glucose) were isolated from urine and plasma, respectively, and their isotopic enrichment determined (described below).

Fractional gluconeogenesis and UDP- gluconeogenesis during fasting were determined using both the glucuronide probe and the acetyl probe. Two separate overnight admissions were necessary. Acetaminophen, sulfamethoxazole and 1-13C acetate were administered during one admission; 2-13C acetate was substituted for 1-13C acetate during the second admission. Metabolites (acetaminophen glucuronide and sulfamethoxazole acetate from urine, and glucose from plasma) were isolated and their isotopic enrichment measured (see below).

Isolation and Determination of Enrichment of Acetaminophen Glucuronide:

Acetaminophen glucuronide was isolated using HPLC (high performance liquid chromatography) (Model 338, Beckman Instruments, Palo Alto, CA). Centrifuged urine aliquots were injected onto a reverse phase C-18 resolve column cartridge (Waters, Inc., Milford, MA) in a radial compression module. The elution buffer was 2% acetonitrile

in water with 1 ml glacial acetic acid per liter at room temperature. UV (ultraviolet) monitoring was at wavelength 254 (Hellerstein, Greenblatt & Munro, 1986). Baseline separated peaks (Figure 13) were collected manually after confirming their identity using acetaminophen glucuronide standard. Collected samples were then lyophilized prior to reconstitution in 100% methanol for HPLC-MS (liquid chromatography-mass spectrometry) analysis.

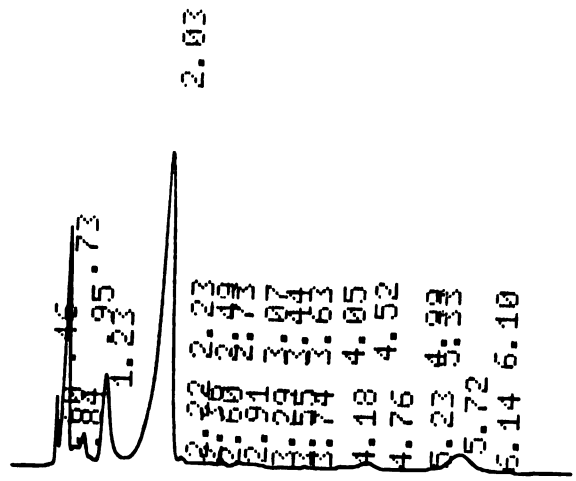
Enrichment of acetaminophen glucuronide was determined using an HPLC-MS system (VG Instruments, Model VG30-250) with elution buffer consisting of methanol: 0.2% ammonium acetate (90:10, v:v). After 1-d1 glucose administration, selective ion recording (SIR) of the molecular anions at 327 and 326 are compared. The standard error of this method was <0.0003 molar excess with 4-6 replicate injections (Hellerstein, Wu, Kaempfer et al., 1989).

Isolation and Determination of Enrichment of Sulfamethoxazole Acetate:

HPLC (see above) was also used to isolate sulfamethoxazole acetate. Aliquots of centrifuged urine were injected onto a reverse phase C-18 microbondapak column cartridge (Waters Inc., Milford, MA) in a radial compression module. The elution buffer was 0.067 M phosphate buffer at pH 3.5 : methanol (65:35, v:v)(Weber & Opheim, 1983). The flow rate was 4.0 ml/min at room temperature with UV monitoring at 225 nm (Kaempfer, Wu, & Hellerstein, 1989).

Figure 13

Elution Profile of Acetaminophen Glucuronide from Human Urine using HPLC (Peak at retention time 2.03)



Baseline separated peaks (Figure 14) were collected manually, using sulfamethoxazole acetate standard (generously provided by Hoffman-LaRoche, Nutley, NJ) to confirm their identity.

Sulfamethoxazole acetate samples were heated in a 55 degree C. water bath (to remove the methanol), lyophilized, then extracted with ethyl acetate and dried under N2. For HPLC-MS analysis samples were reconstituted in 60% methanol in water. The HPLC-MS system and elution buffer were the same as those used to determine acetaminophen glucuronide enrichment. Selective ion recording of the molecular anions at 295 and 294 are compared after administration of 1-13C or 2-13C acetate (Figure 15). The standard error of this method was <0.0003 molar excess (Wu, Kaempfer, Reid et al., 1989).

Isolation and Determination of Enrichment of Plasma Glucose:

Plasma was deproteinized using 0.3 M barium hydroxide and 4% zinc sulfate. Plasma glucose was isolated by passing the plasma supernatant sequentially through anion (Biorad AG 1-X8, formate form, 100-200 mesh) and cation (Biorad AG 50W-X8, hydrogen form, 100-200 mesh) exchange columns, then eluting with water. The eluant was lyophilized, then reconstituted in 100% methanol for HPLC-MS analysis. Selective ion recording was performed of the molecular anions at 180 and 179. The HPLC-MS system and elution buffer were the same as that used for acetaminophen

Figure 14

Elution Profile of Sulfamethoxazole Acetate from Human Urine using HPLC (Peak at retention time 5.68)

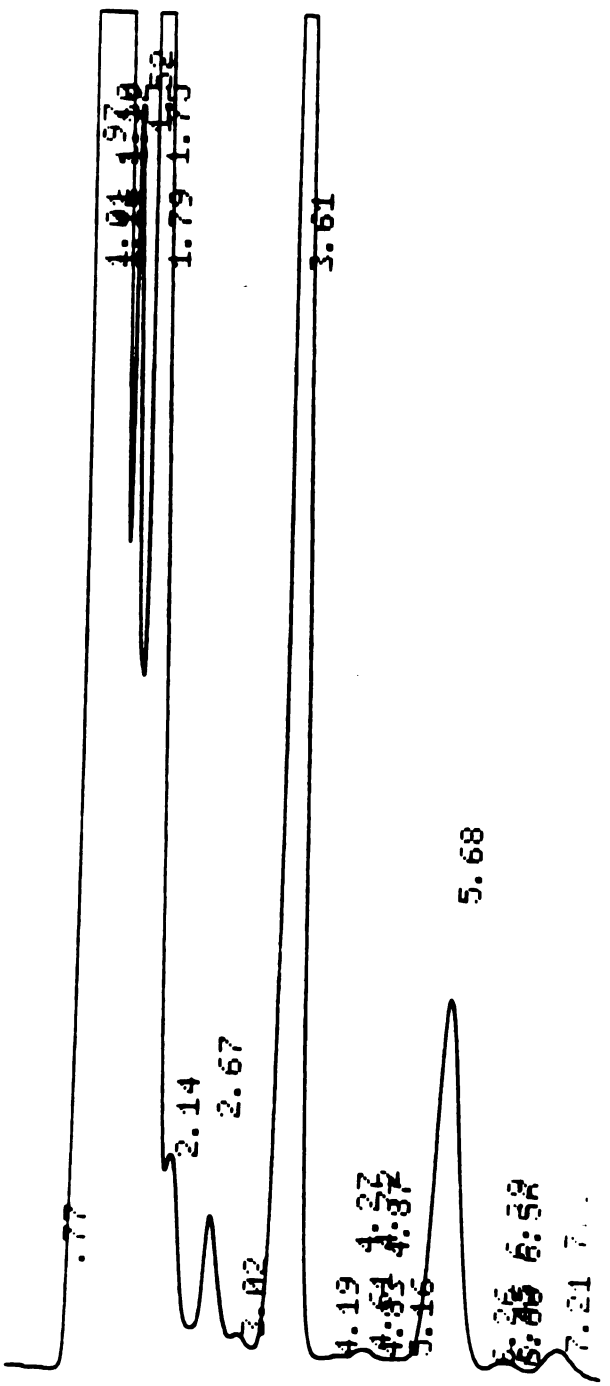
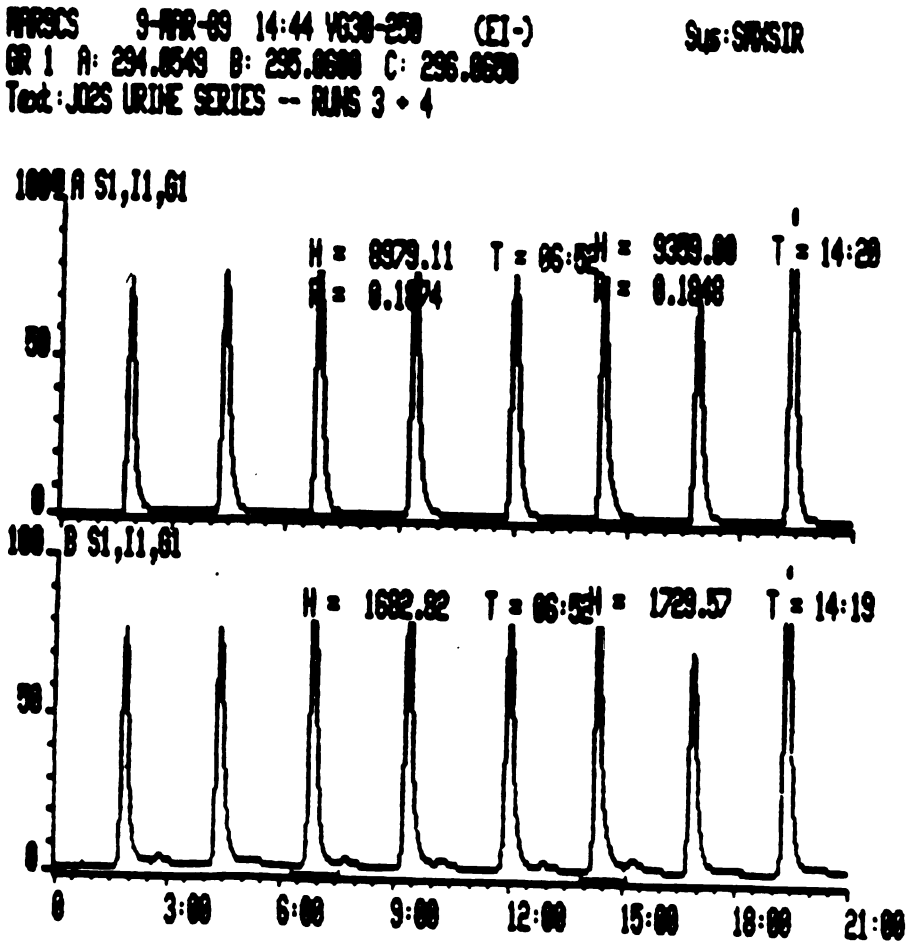


Figure 15

Representative Selective Isotope Recording, using HPLC-MS, of Sulfamethoxazole Acetate Isolated from Human Urine



glucuronide and sulfamethoxazole acetate and the standard error was similar (<0.0002 molar excess).

Procedure

Subjects underwent one baseline evaluation as an outpatient, followed by one inpatient admission (Admission #1) to determine pathway of hepatic glucose utilization. The subset of subjects in whom fractional gluconeogenesis and UDP- gluconeogenesis were to be determined consisted of subjects who were willing to undergo two additional inpatient admissions (Admissions # 2 and 3). All three admissions were separated by one week to allow isotope clearance. The protocols for the two additional admissions were identical except one involved infusion of 1-13C acetate and the other involved infusion of 2-13C acetate. The study protocol was as follows:

Baseline (day 0): A history and physical (including current body weight) was performed by a physician. Subjects received 500 mg. of sulfamethoxazole P.O. and 650 mg. acetaminophen P.O. and were asked to collect urine over the next 4 hours to serve as baseline samples.

Admission #1:

day 1:

18:00 Admit to General Clinical Research Center. Begin NPO except non-caloric beverages (no caffeine or cigarettes allowed)

20:00 Placement of intravenous line

day 2:

02:00 Begin constant infusion of 1-d1 glucose (0.06 mg/kg/min) and acetaminophen (100 mg/hr). Collect urine aliquots through 18:00

08:00 Begin hourly collection of blood samples (from heparin lock)

09:00 Begin hourly ensure feeding (3.1 ml/kg/hr) or intravenous glucose (7 mg/kg/hour) constant infusion until completion of study

18:00 Completion of study

Admission #2 (one week later) involving only the subset of subjects:

day 1:

18:00 Admit to General Clinical Research Center. Begin NPO except non-caloric beverages (no caffeine or cigarettes allowed)

20:00 Placement of intravenous line

day 2:

12:00 Sulfamethoxazole 750 mg. P.O.

02:00 Begin constant infusion of 2-13C acetate (4 mg/kg/hr) and acetaminophen (100 mg/hr)

04:00 Sulfamethoxazole 500 mg. P.O.

10:00 Completion of Study

Admission #3 (One week later) involving only the subset of subjects: (Same as Admission #2, substituting 1-13C acetate, 6 mg/kg/hr, for 2-13C acetate)

All baseline outpatient evaluations and all inpatient metabolic studies were conducted at the General Clinical Research Center, San Francisco General Hospital. Isolation of metabolites was performed in the laboratory of Dr. Marc Hellerstein at the Department of Nutritional Sciences, University of California, Berkeley. HPLC-MS was performed in the laboratory of Dr. Cedric Shackleton at Oakland Children's Hospital.

Limitations

There were several technical limitations encountered during this study. These included an allergic reaction to the sulfamethoxazole acetate, intolerance of ensure feedings, and febrile responses. Difficulties were encountered in recruiting subjects who were willing to participate in all three study admissions. Subject attrition also prevented the completion of both arms of all of the acetate infusion studies. Thus sample size limited the magnitude of effect sizes observed for acetate incorporation into plasma glucose and UDP-glucose. In addition, the sample size was insufficient to give an indication of the variability within or between subjects on these parameters.

The major methodologic limitation of the present study was the necessity for two separate isotopic infusions (1-13C acetate and 2-13C acetate), separated by a week, to determine fractional gluconeogenesis and UDP-gluconeogenesis in the subset of subjects. To the extent

that experimental conditions (i.e. the metabolic state of the subject) differ between the times of the two infusions, error variance may have been introduced into measurements. Currently, the only way to administer concurrent isotopes of acetate labeled in the one and two position would be to use one stable isotope of acetate and one radioactive isotope of acetate, which is ethically unacceptable in humans.

Data Analysis Plan

The methods employed in the present study involved original research in humans, therefore effect sizes for evaluating differences between groups have not yet been determined. Accordingly, the group data in this study were analyzed descriptively, in the form of ranges, means and standard deviations, which can be used in future research requiring estimations of effect size.

Chapter IV

Results

Overview

The study results are presented in three sections. The characteristics of the sample of twenty-seven subjects are first described. In the second section, the first research question (what is the hepatic glucose utilization pathway during fasting and subsequent refeeding in individuals representing a range of HIV infection and weight loss as compared with healthy controls?) is addressed. Fraction of direct glucose pathway during fasting and during refeeding are presented for individuals and then summarized for groups. These data are followed by calculated effect sizes for pairwise intergroup comparisons. Findings related to the second research question (what is the nature of hepatic gluconeogenesis and UDP-gluconeogenesis during asymptomatic HIV infection?) are covered in the third section. Plateau enrichments of acetaminophen glucuronide, sulfamethoxazole acetate, and plasma glucose from 1-13C acetate and 2-13C acetate and incorporation ratios derived from these data are presented for individual subjects in each group. These values are used to determine effect sizes for pairwise intergroup comparisons of incorporation of 13C label into plasma glucose and UDP-glucose. Problems encountered in determining fraction of hepatic gluconeogenesis and UDP-gluconeogenesis from phosphoenolpyruvate during fasting are

then summarized.

Characteristics of the Sample

The total study sample consisted of twenty-seven adult males, ranging in age from nineteen to fifty-six years. One was Black, three were Hispanic, and the remainder were Caucasian. Table 10 displays descriptive data for the entire study sample by group.

Characteristics of the Group of Healthy Volunteers

There were twelve healthy volunteers in the study, eleven Caucasian and one Hispanic (See Tables 10 and 11). They ranged in age from nineteen to fifty-five years with a mean and standard deviation of 38.08 and 11.05, respectively. The mean body weight for this group was 73.66 kg. (s.d. 16.98), and all subjects were 100% of usual weight. The healthy volunteers were verified to be HIV negative by serological testing.

Characteristics of the Asymptomatic HIV Positive Group

Seven subjects who were HIV positive but experiencing no symptoms of the disease were included in the study (See Tables 10 and 11). This group ranged in age from 23 to fifty-six years (mean, 37.0; s.d., 10.65), and consisted of five Caucasians, one Hispanic, and one Black, none of whom was taking AZT. The mean body weight of this group was 73.0 kg., with a s.d. of 12.2 and all subjects were 100% of usual body weight. Potential participants for this group (who fulfilled subject criteria) tended to be employed and

Table 10.

Description of Study Sample by Subject

	Subject I.D.	Race	Weight (Kg.)	% Usual Weight	Age
Healthy Volunteer					
	SC	C	98.8	100	43
	AE	C	75.9	100	19
	UF	C	70.6	100	21
	RH	C	65.3	100	46
	MH	C	64.0	100	40
	BH	C	58.0	100	46
	FK	C	96.0	100	43
	HM	H	52.1	100	24
	KM	C	88.0	100	41
	JO	C	57.3	100	42
	BR	C	96.6	100	55
	MKH	C	61.3	100	37
Asympto-matic HIV					
	LC	C	84.9	100	41
	RM	C	51.7	100	34
	TP	C	73.2	100	41
	TR	H	70.4	100	28
	RT	C	79.0	100	36
	AG	C	86.8	100	56
	DW	B	65.0	100	23

Table 10.

Description of Study Sample by Subject (continued)

	Subject I.D.	Race	Weight (Kg.)	% Usual Weight	Age
AIDS					
	PF*	C	65.4	90	27
	RDM	C	78.8	83	50
	RV	C	70.0	81	42
	RL*	H	57.5	83	37
	AB	C	61.5	91	39
	WM	C	63.6	80	30
	KA*	C	55.0	80	34
	JB*	C	55.2	83	45

Note. I.D.= identification; Kg.= kilgram; C= caucasian; B= black; H= hispanic; *= currently losing weight

Table 11.

Summary Statistics by Group on Descriptive Variables.

	Age (years)	Weight (kg.)	%Usual Weight
Healthy Volunteers (n=12)			
range	19-55	52.1-98.8	---
mean	38.08	73.66	100
s.d.	11.05	16.98	---
Asymptomatic HIV (n=7)			
range	23-56	51.7-86.8	---
mean	37.0	73.0	100
s.d.	10.65	12.20	---
AIDS (n=8)			
range	27-50	55.0-78.8	80-91
mean	38.0	63.38	83.88
s.d.	7.67	8.12	4.29

were therefore more difficult to schedule for the inpatient admissions than the AIDS group.

Characteristics of the AIDS Group

Eight subjects who met the CDC definition of AIDS participated in the study (see Tables 10 and 11). (One subject, KA, did not have a history of opportunistic infection but had constitutional symptoms and more than 10% body weight loss as his AIDS defining diagnosis). The mean age of this group was thirty-eight years (s.d., 7.67), and ranged from twenty-seven to fifty years. In this group, weight ranged from 55.0 to 78.8 kg. (mean, 63.38; s.d., 8.12) The AIDS group consisted of 7 Caucasians and one Hispanic. All subjects in this group had experienced weight loss. For the group as a whole, mean % usual body weight was 83.88% (s.d. 4.29).

For data analysis, subjects in the AIDS group were further classified according to whether or not they were currently losing weight at the time of study participation. Three subjects were clinically stable and not currently on a weight losing trajectory: RDM and RV (both of whom had KS) and AB. Among the catabolic subjects, two (PF and JB) experienced febrile episodes during study glucose pathway protocols. Three subjects had histories of recent opportunistic infections (PF, RL, and WM). Four subjects experienced either intermittent or chronic diarrhea (PF, RL, KA, and WM). All but three subjects were currently taking

AZT, and the remainder had used AZT in the past (PF was paraplegic as a result of AZT neuropathy).

Findings Related to the Research Questions

Pathway of Hepatic Glucose Utilization

The first research question (what is the hepatic glucose utilization pathway during fasting and subsequent refeeding in individuals representing a range of HIV infection and weight loss as compared with healthy controls) was examined using the glucuronide probe. The pathway of hepatic glucose utilization was determined during conditions of steady state fasting and refeeding with IV acetaminophen and 1-d1 glucose. The ratio of plateau acetaminophen glucuronide enrichment to plasma glucose enrichment from 1-d1 glucose yields the fraction direct pathway for glucose entry into hepatic UDP-glucose.

Not all subjects participated in this portion of the study: 9 out of 12 subjects in the healthy volunteer group, 7 out of 7 in the asymptomatic HIV group, and 7 out of 8 in the AIDS group. In addition, not all subjects provided values for both fasting and refeeding for the following reasons. Three subjects (BH, a healthy volunteer; and TR and AG, asymptomatic HIV subjects) only had plateau data for refeeding, either because delays in administering drug and label precluded the collection of plateau fasting urine and blood samples, due to difficulty establishing venous access, or due to inadvertent omission of urine collections. Five

subjects (JO and HM, healthy volunteers; DW, asymptomatic HIV subject; and RV and RDM, AIDS subjects) produced plateau fasting values only, because they were refed with breakfast (bolus feeding), which does not produce a physiological steady state of refeeding. Five subjects selected from all three groups were refed with IV glucose (KM, a healthy volunteer; TR and AG, who had asymptomatic HIV, and PF and RL, AIDS subjects who refused ensure due to their history of diarrhea). The data are presented both for oral refed subjects and combined oral and IV glucose refed subjects.

Fraction of direct pathway of hepatic glucose utilization is presented in Tables 12, 13, and 14, and in figure 16. All three groups of subjects in this part of the study demonstrated predominantly indirect hepatic glucose uptake during fasting. Among the healthy volunteers (n=8 of 9), fraction of direct glucose utilization during fasting ranged from 0.079 to 0.357 (with a mean of .189 and s.d. of .108). During fasting, asymptomatic HIV subjects (n=5 of 7) showed a similar fraction of direct glucose utilization to that of healthy volunteers (mean .190, s.d. .152, range .015 to .391). Subjects with AIDS (n=7 of 7), like the other two groups, predominantly used the indirect glucose pathway during fasting. The mean fraction direct pathway was .09 (s.d. .072). When catabolic AIDS subjects were considered separately (n=4 of 7), this value was even lower (mean, .043, s.d. .036). The fraction direct pathway during both

Table 12.

Fraction of Direct Hepatic Glucose Utilization (FracDir) During Fasting by Subject within Groups

Healthy Volunteer		Aymptomatic HIV		AIDS	
Subject I.D.	Frac Dir	Subject I.D.	Frac Dir	Subject I.D.	Frac Dir
SC	.27/ 1.748 =.154	LC	.43/ 2.27 =.189	RDM	.26/ 2.01 =.129
AE	.33/ .97 =.34	DW	.04/ 2.605 =.015	RV	.53/ 2.44 =.217
UF	.08/ .855 =.094	RT	.12/ 1.61 =.075	PF	.02/ .73 =.027
MH	.25/ .70 =.357	TP	.50/ 1.775 =.282	RL	.125/ 2.05 =.061
BR	.33/ 1.59 =.208	RM	.77/ 1.97 =.391	AB	.18/ 1.60 =.113
JO	.32/ 1.78 =.180			KA	.08/ .98= .082
HM	.17/ 2.16 =.079			JB	0.0/ 1.58= 0.0
KM	.11/ 1.12 =.098				

Note. Plateau acetaminophen glucuronide enrichment/ plateau plasma glucose enrichment = fraction direct pathway

Table 13.

Fraction of Direct Hepatic Glucose Utilization (FracDir) During Refeeding by Subject within Groups

Healthy Volunteer		Asymptomatic HIV		AIDS	
Subject I.D.	Frac Dir	Subject I.D.	Frac Dir	Subject I.D.	Frac Dir
SC	.33/ .62 =.532	LC	.36/ .697 =.516	PF	.15/ .35 =.429
AE	.285/ .398 =.716	RM	.703/ 1.1 =.639	RL	.335/ .753 =.445
UF	.24/ .385 =.623	RT	.175/ .573 =.305	AB	.123/ .517 =.238
MH	.21/ .245 =.857	TR	.354/ .731 =.484	KA	.0967/ .57= .170
BR	.285/ .340 =.838	TP	.317/ .642 =.494	JB	0.0/ .707= 0.0
BH	.555/ .88 =.631	AG	.427/ .75 =.569		
KM	.33/ .69 =.478				

Note. Plateau acetaminophen glucuronide enrichment/ plateau plasma glucose enrichment = fraction direct pathway

Table 14.

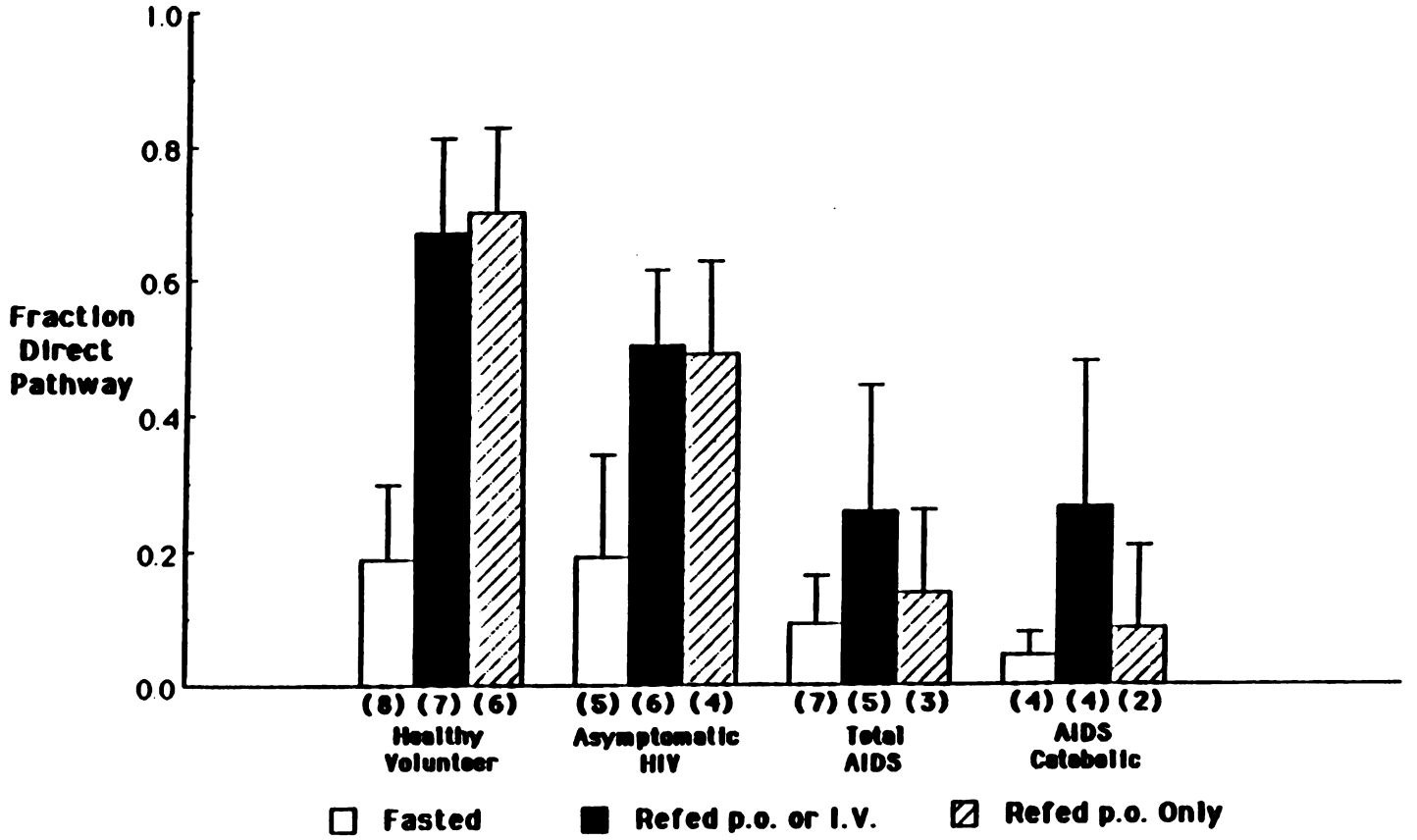
Fraction of Direct Hepatic Glucose Utilization During Fasting and Refeeding by Group

	Healthy Volunteer	Asympto-matic HIV	Total AIDS	Catabolic AIDS
Fasted				
n	8	5	7	4
range	.079-.357	.015-.391	0-.217	0-.082
mean	.189	.190	.090	.043
s.d.	.108	.152	.072	.036
Refed: P.O. or I.V.				
n	7	6	5	4
range	.478-.857	.305-.639	0-.445	0-.445
mean	.668	.501	.256	.261
s.d.	.144	.112	.186	.215
Refed: P.O. only				
n	6	4	3	2
range	.532-.857	.305-.639	0-.238	0-.170
mean	.700	.489	.136	.085
s.d.	.129	.138	.123	.12

Note. Plateau acetaminophen glucuronide enrichment / plateau plasma glucose enrichment = fraction direct pathway

Figure 16

Fraction of Direct Hepatic Glucose Utilization During Fasting and Refeeding



fasting and refeeding for one AIDS subject (JB) was so low that it exceeded the ability of HPLC-MS to distinguish differences in enrichments from baseline values (i.e. his enrichments were less than .05 at both plateaus). The values for fasted and refed plateau enrichments for this subject have thus all been recorded as zero, implying an entirely indirect (recycled) pathway, regardless of feeding state.

Patterns suggestive of potential group differences emerged when fraction of direct hepatic glucose utilization in response to feeding was examined. During plateau refeeding, regardless of feeding route, healthy volunteers (n=7 of 9) and subjects with asymptomatic HIV infection (n=6 of 7) tended to switch to predominantly direct pathway utilization (mean .668, s.d. .144 and mean .501, s.d. .112, respectively for both feeding routes; mean .700, s.d. .129 and mean .489, s.d. .138, respectively for oral feeding alone).

In contrast, the AIDS group (n=5 of 7) demonstrated greater variability in response to refeeding. In this group, no subjects changed to predominantly direct hepatic glucose utilization. Thus there appeared to be a trend whereby the AIDS group as a whole showed less fraction direct pathway than either of the other two groups (means of .256 and .261, for the total AIDS group (n=5 of 7) and catabolic AIDS subgroup (n=4 of 7), respectively). This

trend became more pronounced when subjects refed by the oral route were considered separately. In the AIDS group (n=3 of 7) , oral refeeding resulted in an increase in fraction direct hepatic glucose utilization to a mean of only .136 (s.d. .123). When only catabolic orally fed subjects (n=2 of 7) were considered, there was practically no increase in fraction direct glucose utilization, to a mean of .085 from .043 during fasting (although the sample size of two limits generalization of this observation).

Healthy volunteers showed a mean increase in direct pathway of 46.55% (s.d. 10.51). This was nearly twice the increase demonstrated by the other groups: mean of 25.45% (s.d. 5.05) in asymptomatic HIV subjects and mean of 19.99% (s.d. 18.22) in the AIDS group.

The foregoing findings are descriptive only, and can not be construed as demonstrating statistically significant group differences. The summary statistics, however, may be used to determine effect sizes for use in statistical power analysis for future hypothesis-testing research. In order to determine the effect of group membership on hepatic glucose utilization pathway, effect sizes for all three possible pairwise group comparisons (healthy volunteer vs. asymptomatic HIV; healthy volunteer vs. AIDS; and asymptomatic HIV vs. AIDS) were performed using the method of Cohen (1977). Effect sizes were calculated separately for fraction direct pathway during fasting and fraction

direct pathway during refeeding, as follows:

$$\text{effect size} = \frac{|\text{mean of group 1} - \text{mean of group 2}|}{\text{average standard deviation of both groups}}$$

The summary data used to calculate effect sizes are found in Table 14. The calculated effect sizes for each comparison are summarized in Table 15.

Conventional values for small, medium, and large effect sizes are 0.2, 0.5, and 0.8, respectively (Cohen, 1977). From Table 15 it can be seen that for all comparisons except one,the effect of group membership is large for fraction of direct pathway. There is no effect of group membership for the comparison of healthy volunteers with asymptomatic HIV subjects on fraction of direct pathway during fasting (effect size of -.008). The remaining comparisons range from .785 to 1.457. These results indicate that clinical state related to HIV infection exerts an effect on pathway of hepatic glucose utilization, and that the method employed in this study to measure glucose pathway is sensitive enough to detect this effect. A Kruskall-Wallis test was performed on the glucose pathway data. The effect of group membership on glucose pathway during refeeding was statistically significant: $H=10.896$, $p<.0043$, with the planned contrast between healthy volunteer and AIDS groups significant at $p<.05$. In a study involving a hypothesis test of actual group differences in hepatic glucose utilization pathway, matching of potential confounding variables between groups

Table 15.

Effect Sizes of Group Membership for Pairwise Comparisons: Fraction Direct Hepatic Glucose Utilization

	Healthy vs. Asymptomatic HIV	Healthy vs. AIDS	Asymptomatic vs. AIDS
Fraction Direct Pathway, Fasting	-.008 (none)	1.1 (large)	.893 (large)
Fraction Direct Pathway, Refed	1.305 (large)	2.5 (large)	1.64 (large)

Note. Direction of effect is expressed according to group order in each pair, i.e. a positive value indicates that the first group had a higher mean, a negative value indicates that the second group had a higher mean

would be desirable, achieved either during subject recruitment or by adjusting for covariates that do not define study groups (such as age or lean body mass) during statistical analysis. In the present sample, age was uncorrelated with fraction direct glucose pathway used by the liver during either fasting or refeeding. In contrast, percent usual body weight (a criterion defining group membership) was highly correlated with fraction direct pathway during refeeding ($r=.7125$, $p=.0009$).

Hepatic Gluconeogenesis and UDP-Gluconeogenesis

The second research question (what is the nature of hepatic gluconeogenesis (GNG) and UDP-gluconeogenesis (UDP-GNG) during asymptomatic HIV infection?) was addressed using both the acetyl and glucuronide probes. These involve administration of oral sulfamethoxazole and infusions of acetaminophen and 13C acetate. Mean incorporation ratios for 1- and 2-13C acetate into plasma glucose and acetaminophen glucuronide are used in calculations of hepatic GNG and UDP-GNG from grouped data. In the present analysis, these ratios are summarized by group, and also used in calculations of effect size for pairwise group comparisons in order to demonstrate the sensitivity of this isotopic method in detecting group effects for isotope incorporation. The error sensitivity of the method (described in detail in Chapter 2) in calculating valid fractional GNG and UDP-GNG from small samples was an

important finding. The results of the analysis indicate that this method is too error sensitive to permit these calculations using the limited sample sizes available for the present study.

Data from a total of 5 out of 12 healthy volunteers were available, two of whom (HM and JO) volunteered to have the two infusions repeated after an interval of more than six months. One subject in this group (RH) developed a hemolytic reaction to the sulfamethoxazole (despite having no known previous history of allergy to sulfonamides), and was only able to complete one of the 13C acetate studies.

The asymptomatic HIV group consisted of 2 out of 7 subjects, only one of whom provided complete data. Subject TR was admitted for both 13C acetate studies, one of which was unusable because a dose of sulfamethoxazole was not given by mistake.

Data from five subjects with AIDS who also underwent infusions on this protocol in a related study were available for analysis. They have been included to provide further information on the applicability and limitations of using non-invasive probes to determine GNG and UDP-GNG. Within the AIDS group, complete data from two infusion protocols were provided for only two subjects (KA and RL). The remaining members of this group provided data from only one infusion, either due to the constraints of the study design (WM and JB) or due to subject attrition (RV).

In the group of healthy volunteers, incorporation ratios for 1-13C acetate into acetaminophen glucuronide ranged from 0.013 to 0.065 (mean .041; s.d. .022)(Table 16). The asymptomatic HIV and AIDS groups showed mean incorporation ratios of .032 (s.d..001) and .089 (s.d. .011), respectively. Mean incorporation ratios for 2-13C acetate into acetaminophen glucuronide, were higher for all three groups (.098 for healthy volunteers, .104 for asymptomatic HIV, and .121 for AIDS)(See Table 17).

Fraction of hepatic gluconeogenesis is determined from incorporation ratios for both 1-13C acetate and 2-13C acetate into plasma glucose (Tables 16 and 17). During infusion of 1-13C acetate, healthy volunteers had a mean incorporation of .046 (s.d. .013); while the two asymptomatic HIV subjects showed a mean incorporation of .042 (s.d. .015) and the AIDS group, a mean of .095 (s.d. .051). Analogous to acetaminophen glucuronide, infusions of 2-13C acetate yielded higher incorporation ratios into plasma glucose for all three groups: a mean of .11 in healthy volunteers (s.d. .021) and a mean of .155 in the AIDS group. Only one value for 2-13C incorporation into plasma glucose was available for the asymptomatic HIV group (.093).

The ratio of 2-13C to 1-13C acetate incorporation is used to calculate fraction of both GNG and UDP-GNG during fasting. Since this is a ratio, small changes in the

Table 16.

Incorporation Ratios and Plateau Enrichments of Acetaminophen Glucuronide (GlucUA), Sulfamethoxazole Acetate (SMX-Ac), and Plasma Glucose (Gluc) from 1-13C Acetate during Fasting by Subject within Groups

	GlucUA / SMX-Ac	Gluc / SMX-AC	GlucUA	SMX-Ac	Gluc
Healthy Volunteer					
FK	.013	.05	.09	7.01	.38
HM	.024	.046	.28	11.66	.54
HM	.049	.050	.55	11.16	.56
JO	.065	.054	.70	10.72	.58
MKH	.053	.024	.398	7.56	.18
Mean	.041	.046			
S.D.	.022	.013			
Asympto-matic HIV					
TR	.033	.053	.32	9.86	.52
AG	.031	.032	.31	10.10	.32
Mean	.032	.042			
S.D.	.001	.015			

Table 16.

Incorporation Ratios and Plateau Enrichments of Acetaminophen Glucuronide (GlucUA), Sulfamethoxazole Acetate (SMX-Ac), and Plasma Glucose (Gluc) from 1-13C Acetate during Fasting by Subject within Groups (continued)

	GlucUA / SMX-Ac	Gluc / SMX-Ac	GlucUA	SMX-Ac	Gluc
AIDS					
KA	.096	.131	.66	6.86	.90
RL	.081	.058	.48	5.91	.345
Mean	.089	.095			
S.D.	.011	.051			

Note. Mean incorporation ratios are used to determine the ratio of 2-13C acetate to 1-13C acetate label incorporation into plasma glucose and acetaminophen glucuronide according to the method of Weinman et al. (1957) as elaborated by Katz (1985)

Table 17.

Incorporation Ratios and Plateau Enrichments of Acetaminophen Glucuronide (GlucUA), Sulfamethoxazole Acetate (SMX-Ac) and Plasma Glucose (Gluc) from 2-13C Acetate during Fasting by Subject within Groups

	GlucUA / SMX-Ac	Gluc / SMX-Ac	GlucUA	SMX-Ac	Gluc
Healthy Volunteer					
FK	.078	.115	.465	5.93	.68
HM	.070	.111	.33	4.73	.527
HM	.126	.135	.69	5.47	.74
JO	.101	.125	.58	5.74	.715
JO	.070	.100	.48	6.88	.69
MKH	.132	.113	.853	6.46	.73
RH	.106	.069	.64	6.06	.42
Mean	.098	.11			
S.D.	.026	.021			
Asympto-matic HIV					
AG	.104	.093	.67	6.47	.60
Mean	.104	.093			
S.D.	-----	-----			

Table 17.

Incorporation Ratios and Plateau Enrichments of Acetaminophen Glucuronide (GlucUA), Sulfamethoxazole Acetate (SMX-Ac) and Plasma Glucose (Gluc) from 2-13C Acetate during Fasting by Subject within Groups (continued)

	GlucUA / SMX-Ac	Gluc / SMX-Ac	GlucUA	SMX-Ac	Gluc
AIDS					
RV	.154	.166	1.08	7.03	1.17
KA	.162	.244	.50	3.08	.75
RL	.082	.103	.48	5.84	.60
JB	.140	.171	.705	5.04	.86
WM	.069	.092	.43	6.21	.57
Mean	.121	.155			
S.D.	.043	.061			

Note. Mean incorporation ratios are used to determine the ratio of 2-13C acetate to 1-13C acetate label incorporation into plasma glucose and acetaminophen glucuronide according to the method of Weinman et al. (1957) as elaborated by Katz (1985)

denominator (1-13C acetate incorporation) can have a large effect on the calculated value. Moreover, the calculations derived from this ratio are very sensitive to small differences, a problem noted more than 30 years ago when the mathematics of the technique were introduced (Weinman, Strisower, & Chaikoff, 1957). Accordingly, even small changes in mean incorporation, produced by an outlier or variability in a small sample can profoundly influence the calculation.

Fractional hepatic gluconeogenesis and UDP-gluconeogenesis indicate the percentage of plasma glucose and UDP-glucose synthesized from PEP, respectively. In this study these parameters were determined (using the method described in Chapter 2) during steady state fasting conditions only in the group of healthy volunteers, because limited sample size within the other two groups raised concerns about the validity of their respective means as estimates of population parameters. From the present data, 38.9% of plasma glucose and 35.4% of UDP-glucose came from PEP during a fast among healthy volunteers. In view of the foregoing concerns, however, these values are probably only semi-quantitative estimates, the validity of which would improve with a larger number of subjects, as indicated by calculations of effect size.

Effect sizes for all possible pairwise group comparisons on 1- and 2-13C acetate into plasma glucose and

acetaminophen glucuronide are summarized in Table 18. Group membership exerted a large effect for eight of the twelve comparisons, in spite of the limitations imposed by having only one or two members in some of the groups which were compared (Tables 16 and 17). Magnitude of large effects ranged from .8 to 9.52.

These results indicate that the source of the error sensitivity is not in the isotopic method, but more likely involves both sample size for the HIV group and the elaboration of the isotopic data in the mathematical model. Given an adequate sample size, the present non-invasive probe is sensitive enough to detect group membership effects. The large effects observed support the validity of this method in future hypothesis testing research (which includes a statistical power analysis) of group differences in hepatic GNG and UDP-GNG.

Summary of Results

All subjects participating in the present investigation demonstrated predominantly indirect (recycled) pathway of hepatic glucose utilization during a fast. With refeeding, the direct pathway of hepatic glucose utilization became more dominant in the healthy volunteer and asymptomatic HIV groups. In contrast, hepatic glucose utilization in the AIDS group continued to be predominantly indirect, with catabolic subjects undergoing oral refeeding showing the most dramatic differences from the other two groups. These

Table 18.

Effect Sizes of Group Membership for Pairwise Comparisons: 13C Acetate Incorporation into Plasma Glucose (Gluc) and Acetaminophen Glucuronide (GlucUA) during Fasting

	Healthy vs. Asymptomatic HIV	Healthy vs. AIDS	Asymptomatic vs. AIDS
1-13C Acetate into GlucUA	.8 (large)	-2.96 (large)	-9.52 (large)
1-13C Acetate into Gluc	.254 (small)	-1.53 (large)	-1.59 (large)
2-13C Acetate into GlucUA	-.248 (small)	-.68 (medium)	-.395 (small)
2-13C Acetate into Gluc	.824 (large)	-1.08 (large)	-1.0 (large)

Note. Direction of effect is expressed according to group order in each pair, i.e. a positive value indicates that the first group had a higher mean, a negative value indicates that the second group had a higher mean

observations coincided with the finding of large effects of group membership in all but one pairwise group comparison for glucose pathway during fasting and during refeeding.

It became clear during the course of the investigation that the 13C acetate method for estimating dilution of 13C acetate in hepatic PEP and thereby calculating hepatic GNG and UDP-GNG is very sensitive to experimental error with small samples. Consequently the experimental data could be fully elaborated, including quantitative estimates of GNG pathway fluxes, for the healthy volunteers only. Thus an answer to the second research question of this study was not able to be provided. However, analysis of the primary data, that is, the isotopic incorporation from 13C acetate into plasma glucose and hepatic UDP-glucose for subjects from all three study groups indicated that this method is sensitive in detecting effects of group membership.

Chapter V

Discussion of the Findings

Overview

The present study described the regulation of hepatic glucose metabolism in a human model of chronic infection, HIV infection. Specifically, the pathway of hepatic glucose utilization during the transition from fasting to refeeding was examined in asymptomatic HIV, AIDS and healthy volunteers. In addition, a method of determining source of plasma glucose and UDP- glucose during fasting was tested.

The research questions were based on a model of mutual inhibition of hepatic glucose and fatty acid oxidation during feeding state transitions. Normally, during a fast fatty acid oxidation and ketone production inhibit glucose (pyruvate) oxidation. Refeeding results in glucose oxidation and lipogenesis, with inhibition of ketogenesis. This pattern of regulation allows the preservation of body protein during periods of caloric insufficiency. The enzyme, pyruvate dehydrogenase (PDH) is considered to be a key regulator of this pattern of glucose and fatty acid metabolism in the liver. Activation of PDH allows pyruvate oxidation and de novo lipogenesis to proceed. PDH inactivation occurs in a setting of net ketogenesis and direction of pyruvate carbon into synthesis of hexose phosphates. A premise of the model is that hepatic metabolic regulation is somehow disturbed during infection,

and is manifested as inhibition of ketogenesis, persistent oxidation of glucose precursors derived from body protein, enhanced de novo lipogenesis, and re-esterification of fatty acids mobilized from the periphery.

Pathway of hepatic glucose utilization was measured in the present study and can be considered an indirect indicator of PDH activation (and, by extension, hepatic glycogen stores), although other factors could conceivably influence glucose pathway (discussed below). Fraction of hepatic gluconeogenesis and UDP-gluconeogenesis from phosphoenolpyruvate (PEP) were not measured in all study groups, however these parameters would provide not only an indication of the fate of pyruvate in the liver, but of the partitioning of hexose phosphate carbon derived from hepatic synthesis (i.e. the activation state of glucose-6-phosphatase)(See Figure 6).

The results of the present study provide a basis for future investigation into the nature of regulation of hepatic glucose metabolism during HIV infection in humans. Fraction of direct hepatic glucose utilization measured using non-invasive probes was found to be affected by clinical state related to HIV infection. Precise quantification of hepatic GNG and UDP-GNG was not possible under the constraints of sample size, cost, and time of the present study. However isotopic incorporation of 13C-acetate into plasma glucose and UDP-glucose also varied

among clinical states. Given the small sample size, the significance of these findings can not yet be definitively stated, but they do invite further study. Attention to several validity issues (discussed below) related to the metabolic model being tested will facilitate the interpretation of future hypothesis tests of group differences in pathway of hepatic glucose utilization or hepatic GNG and UDP-GNG.

Validity Issues

Pathway of Hepatic Glucose Utilization

Fraction of direct hepatic glucose utilization can be influenced by several factors. These include: glucose malabsorption, increased glucose metabolism by enterocytes, PDH activation state, plasma glycemia, insulin action, inhibition of gluconeogenesis, or depletion of hepatic glycogen stores.

Glucose malabsorption would result in reduced availability of intact glucose to the liver. Metabolism of glucose to trioses by enterocytes, due to involvement of these cells by HIV, superimposed infections, or malignancy, or due to damage from antineoplastic chemotherapy would result in increased delivery of trioses to the liver. Malabsorption is a common sequel to AIDS, and occurs even in the absence of diarrhea. In the present study, three catabolic subjects who were refed had histories of diarrhea and one subject (JB) was receiving bleomycin and vinblastine

for KS. Two AIDS subjects received IV glucose refeeding due to concerns about provoking diarrhea by ensure feeding. None of the subjects, however, experienced diarrhea during the course of an infusion. Thus, in the present study, occult glucose (as distinct from fat) malabsorption might to a limited extent account for differences in hepatic glucose utilization and is a variable that probably should be evaluated, if possible, in future studies. (This information would substantiate other data which suggest that activation of hepatic lipogenesis upon ensure feeding occurs, consistent with the conclusion that carbohydrate is absorbed in the AIDS population)(M. K. Hellerstein, 1990, Unpublished raw data).

Phosphorylation state of PDH (one indicator of PDH activation) is readily measured in animal models using an enzyme assay of liver tissue, whereas direct assay of hepatic PDH activity is currently not possible in human studies. PDH activation can, however, be manipulated using pharmacologic interventions such as DCA (dichloroacetate), an activator of PDH. DCA used in conjunction with isotopic studies may provide additional characterization of hepatic substrate regulation, especially if results in humans corroborate animal studies involving enzyme assays of PDH activity.

Magnitude of plasma glycemia could enhance the direct glucose pathway through increased substrate availability for

glucokinase, through provision of triose (pyruvate) for PDH, or via insulin action. To date, however, no correlation has been demonstrated between plasma glucose concentration in the normal range and fraction of direct hepatic glucose utilization during either fasting or refeeding in humans (Hellerstein, Wu, Kaempfer et al., 1989). Thus it is possible that glycemia exerts a direct effect on hepatic glucose utilization pathway only at supraphysiologic glucose concentrations (Lang, Bagby, Blakesley, et al., 1986), which would not be achieved with the methodology employed in the present study.

Insulin has many effects on hepatic metabolic regulation. Several which are relevant to the mutual inhibition of hepatic glucose and fatty acid metabolism are the following: 1) activation of acetyl CoA carboxylase (malonyl CoA synthesis) and thereby inhibition of CPT (and ketone synthesis) with activation of fatty acid and triglyceride synthesis; 2) inhibition of gluconeogenesis, presumably by altering pyruvate kinase and phosphofructokinase cycling; and 3) activation of glycogen synthase and inhibition of glycogenolysis. Whether or not insulin is the principle regulator of substrate flux during feeding state transitions, however, is unknown and has yet to be demonstrated even in animal models (Holness, French, & Sugden, 1986; Holness & Sugden, 1989). Of potential utility in clarifying this question in human studies, will be

isotopic studies in conjunction with glucose , tolbutamide, and insulin clamps, which permit manipulation of circulating insulin and glucose levels for up to several hours, and can be used to infer regulatory effects of insulin on substrate metabolism.

Inhibition of gluconeogenesis would result in a decrease in the indirect (recycled) pathway of hepatic glucose utilization, in part because glucose precursors (trioses) would be diverted into other metabolic pathways such as de novo lipogenesis. In contrast, depletion of (or inability to replete) hepatic glycogen stores would be manifested by persistent indirect hepatic glucose uptake. (Hepatic glycogen repletion results in a relative increase in direct hepatic glucose uptake, although the mechanisms for signalling this switch are still unknown.) Alterations in hepatic glycogen metabolism have been documented in animal models of infection (See Table 8). Using the isotopic methods employed in the present study, fraction of direct hepatic glucose uptake was found to be correlated with degree of hepatic glycogen repletion in rats (Hellerstein, Greenblatt, & Munro, 1986). Hepatic glycogen stores cannot be assayed directly in humans, however use of 1-d1 galactose in conjunction with the glucuronide probe could be used to determine rate of appearance of UDP-glucose as one index of hepatic glycogen status in relation to hepatic glucose utilization pathway during HIV infection.

Of particular interest, given the results of the present study, would be whether hepatic glycogen depletion paradoxically coincides with enhanced de novo lipogenesis during HIV infection, since this would represent an anomalous or dysregulatory combination.

Measurement of Hepatic GNG and UDP-GNG

Three major sources of error variance are inherent to the measurement of hepatic GNG and UDP-GNG using labelled acetate: metabolic, experimental, and mathematical. Using acetate to label the products of hepatic GNG and UDP-GNG is in part confounded by the variety of potential fates of labeled acetate carbon and the inability to exactly quantitate them. These fates include peripheral metabolism of acetate with subsequent reincorporation of labeled CO2 into gluconeogenic precursors (e.g. alanine and glutamine), or into hepatic oxaloacetate via pyruvate carboxylase. Pyruvate kinase cycling is another potential source of error. Despite these problems, acetate remains the current label of choice for measuring GNG (Consoli, Kennedy, Miles et al., 1987; Consoli, Nurjhan, Capani et al., 1989).

On the experimental level, the necessity of two separate infusions, separated by a week, introduces variability into the measurement of hepatic GNG and UDP-GNG although the assumption is made that subjects will be similar metabolically at both points in time. To the extent that this assumption is inaccurate, deviations are assumed

to be randomly distributed in a sample of subjects, therefore determining hepatic GNG and UDP-GNG in a group of subjects decreases error variance. On repeated measurement, two normal volunteers (HM and JO) demonstrated intrasubject variability in plateau enrichments of metabolites and in 13C incorporation ratios. This illustrates the measurement error involved in determining hepatic GNG or UDP-GNG using data from single subjects or small samples. The numbers of subjects which have been studied thus far using the methodology described in the present study, though, is still insufficient to indicate the magnitude of either the inter- or intrasubject variability involved in measuring hepatic GNG and UDP-GNG, and therefore the numbers of observations required to validly determine these parameters.

The major source of error sensitivity in determining hepatic GNG and UDP-GNG using infusions of 1- and 2-13C acetate is the mathematical model used to derive these parameters from isotopic incorporation data (Katz, 1985; Weinman, Strisower, & Chaikoff, 1957). In these equations, even small changes in the ratio of 2-13C to 1-13C acetate incorporation cause large differences in the final calculation. With a very small sample, the ratios can not even be elaborated in the equations (i.e. the calculations yield mathematically impossible results). In the present study, valid measurement of hepatic GNG and UDP-GNG during fasting was not possible in all of the study groups, because

the isotopic data in the two HIV-infected groups were not compatible with the model, probably for all of the foregoing reasons. (However, the raw incorporation data can legitimately be interpreted for semi-quantitative results.) In view of this unresolved limitation in the HIV positive groups, this method is probably at present best considered to be semi-quantitative in studies involving limited samples.

Relevance to Nursing

Nursing care of patients suffering from HIV infection and AIDS requires an understanding of the pathophysiology of clinical problems, such as weight loss and wasting. Traditionally, weight loss in catabolic patients has prompted attempts to provide nutritional interventions, despite the lack of understanding of the fate of administered nutrients in this setting. Provision of substrate, in the form of calories and protein, has, however, thus far been ineffective in reversing the trajectory of weight loss in AIDS and other wasting illnesses. The possible role of altered metabolic regulation of substrate pathways, therefore, has assumed increasing importance as a focus of clinical investigation.

The present study provides important groundwork for future understanding of the intracellular mechanisms that govern weight loss during HIV infection. Given the necessary invasiveness and expense of the interventions, and

the highly technical nature of the methods employed to analyze clinical samples, it is incumbent upon nursing researchers to continue to participate in such research in a collaborative capacity with physicians, biochemists and other disciplines in the basic sciences. The insights thus gained will provide a basis for developing more effective nursing interventions in this and potentially other patient populations.

Implications for Future Study

The findings of the present study demonstrate both the utility of non-invasive probes in describing hepatic metabolism in HIV infected persons, and also the sensitivity of these methods in demonstrating effect sizes for intergroup comparisons. A number of avenues of future research in this patient population, therefore, seem warranted. These include characterization of other hepatic metabolic pathways, such as de novo lipogenesis, using sulfamethoxazole acetate (acetyl CoA) as precursor and VLDL (very low density lipoprotein) triglyceride (which is synthesized exclusively by hepatocytes) as product. Development of additional probes of acetyl CoA, using drugs that are acetylated but which are less allergenic than sulfamethoxazole would broaden the applicability of this methodology.

Intervention studies have assumed increasing importance in AIDS research. For example, megestrol acetate is

currently being studied as a possible means of reversing HIV related weight loss and wasting, despite the lack of understanding of the exact mechanism by which it exerts any physiological effects (Tchekmedyian, Tait, Moody et al., 1987; VonRoenn, Murphy, Weber et al., 1988). Studies of agents whose biochemical site of action are known might, however, yield a better understanding of the pathophysiology of HIV-related weight loss. For example, by exploiting what is known about pathways of cytokine metabolism (such as the cyclooxygenase metabolism of interleukin-1), clinical trials employing non-invasive probes and agents whose site of action is known (such as fish oils or ibuprofen, which alter prostaglandin synthesis, hydrazine sulfate, which is thought to inhibit gluconeogenesis, or dichloroacetate, which activates hepatic pyruvate dehydrogenase), more detailed characterization of the site of regulatory defects in hepatic metabolism can be determined. Such studies, conducted in conjunction with thorough evaluation of nutritional status and careful assignment of subjects to groups representing degrees of HIV infection may help determine at what point in the course of HIV infection regulatory disturbances begin, and when these alterations may be amenable to prophylaxis and/or treatment.

References

Akerlund, B., Carlson, L. A., & Jarstrand, C. (1986). Dyslipoproteinemia in patients with severe bacterial infections. Scandinavian Journal of Infectious Disease, 18, 539-545.

Ballard, F. J. (1972). Supply and utilization of acetate in mammals. The American Journal of Clinical Nutrition, 25, 773-779.

Bartlett, J. G., Laughon, B., & Quinn, T. C. (1988). Gastrointestinal complications of AIDS. In V. T. DeVita, Jr., S. Hellman, & S. A. Rosenberg (Eds.) AIDS: Etiology, diagnosis, treatment, and prevention (2nd Edition)(pp. 227-244) Philadelphia: J. B. Lippincott.

Beach, R. S., & Laura, P. F. (1983). Nutrition and the Acquired Immunodeficiency Syndrome. Annals of Internal Medicine, 99(4), 565-566.

Beisel, W. R. (1966). Effect of infection on human protein metabolism. Federation Proceedings, 25, 1682-1687.

Beisel, W. R. (1977a). Impact of infection on nutritional status: definition of the problem and objectives of the Workshop. The American Journal of Clinical Nutrition, 30, 1206-1210.

Beisel, W. R. (1972). Interrelated changes in host metabolism during generalized infectious illness. The American Journal of Clinical Nutrition, 25, 1254-1260.

Beisel, W. R. (1977b). Magnitude of the host nutritional

responses to infection. The American Journal of Clinical Nutrition, 30, 1236-1247.

Beisel, W. R. (1975). Metabolic response to infection. Annual Review of Medicine, 26, 9-20.

Beisel, W. R., & Fiser, R. H. (1970). Lipid metabolism during infectious illness. The American Journal of Clinical Nutrition, 23(8), 1069-1079.

Beisel, W. R., Sawyer, W. D., Ryll, E. D., & Crozier, D. (1967). Metabolic effects of intracellular infections in man. Annals of Internal Medicine, 67(4), 744-779.

Berkelman, R. L., Heyward, W. L., Stehr-Green, J. K., & Curran, J. W. (1989). Epidemiology of human immunodeficiency virus infection and acquired immunodeficiency syndrome. The American Journal of Medicine, 86, 761-770.

Berman, M. A., Sandborg, C. I., Calabia, B. S., Andrews, B. S., & Friou, G. J. (1987). Interleukin-1 inhibitor masks high interleukin-1 production in acquired immunodeficiency syndrome (AIDS). Clinical Immunology and Immunopathology, 42, 133-140.

Berry, L. J., Smythe, D. S., & Young, L. G. (1959). Effects of bacterial endotoxin on metabolism. I. Carbohydrate depletion and the protective role of cortisone. Journal of Experimental Medicine, 110, 389-405.

Beutler, B. A., & Cerami, A. (1985). Recombinant interleukin-1 suppresses lipoprotein lipase activity in

3T3-L1 cells. Journal of Immunology, 135(6), 3969-3971.

Blackham, M. Kaempfer, S., Wu, K., Christiansen, M., Vary, T., & Hellerstein, M. (1990). Regulation of hepatic carbohydrate metabolism by nutrients: correlation between pyruvate dehydrogenase (PDH) activity and non-invasive isotopic fluxes. FASEB Journal, 4, A282.

Border, J. R. (1970). Metabolic response to short-term starvation, sepsis, and trauma. Surgery Annual, 2, 11-34.

Brosnan, J. T. (1982). Pathways of carbon flux in gluconeogenesis. Federation Proceedings, 41, 91-95.

Cahill, G. F. (1970). Starvation in man. The New England Journal of Medicine, 282(12), 668-675.

Cannon, J. G., Tompkins, R. G., Gelfand, J. A., Michie, H. R., Stanford, G. G., van der Meer, J. W. M., Endres, S., Lonnemann, G., Corsetti, J., Chernow, B., Wilmore, D. W., Wolff, S. M., Burke, J. F., & Dinarello, C. A. (1990). Circulating interleukin-1 and tumor necrosis factor in septic shock and experimental endotoxin fever. The Journal of Infectious Disease, 161, 79-84.

Canonico, P. G., Ayala, E., Rill, W. L., & Little, J. S. (1977). Effects of pneumococcal infection on rat liver microsomal enzymes and lipogenesis by isolated hepatocytes. The American Journal of Clinical Nutrition, 30, 1359-1363.

Centers for Disease Control, U. S. Department of Health and

Human Services. (1986). Classification system for human t-lymphotropic virus type III/ lymphadenopathy-associated virus infections. Annals of Internal Medicine, 105, 234-237.

Chlebowski, R. T. (1985). Significance of altered nutritional status in acquired immune deficiency syndrome (AIDS). Nutrition and Cancer, 7(1&2), 85-91.

Cohen, J. (1977). Statistical power analysis for the behavioral sciences. Revised edition. New York: Academic Press.

Colman, N., & Grossman, F. (1987). Nutritional factors in epidemic Kaposi's Sarcoma. Seminars in Oncology, 14(2), Suppl.3, 54-62.

Consoli, A., Kennedy, F., Miles, J., & Gerich, J. (1987). Determination of Krebs cycle metabolic carbon exchange in vivo and its use to estimate the individual contributions of gluconeogenesis and glycogenolysis to overall glucose output in man. Journal of Clinical Investigation, 80, 1303-1310.

Consoli, A., Nurjhan, N., Capani, F., & Gerich, J. (1989). Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. Diabetes, 38, 550-557.

Crocker, K. S. (1989). Gastrointestinal manifestations of the acquired immunodeficiency syndrome. Nursing Clinics of North America, 24(2), 395-406.

Curnow, R. T., Rayfield, E. J., George, D. T., Zenser, T. V., & DeRubertis, F. R. (1976). Altered hepatic glycogen metabolism and glucoregulatory hormones during sepsis. American Journal of Physiology, 230(5), 1296-1301.

Dinarello, C. A. (1988). Biology of interleukin-1. FASEB Journal, 2, 108-115.

Dinarello, C. A. (1987). The biology of interleukin-1 and comparison to tumor necrosis factor. Immunology Letters, 16, 227-232.

Dinarello, C. A. (1984a). Interleukin-1. Reviews of Infectious Diseases,6(1), 51-95.

Dinarello, C. A. (1984b). Interleukin-1 and the pathogenesis of the acute-phase response. The New England Journal of Medicine, 311(22), 1413-1418.

Dworkin, B., Wormser, G. P., Rosenthal, W. S., Heier, S. K., Braunstein, M., Weiss, L., Jankowski, R., Levy, D., & Weiselberg, S. (1985). Gastrointestinal manifestations of the Acquired Immunodeficiency Syndrome: A review of 22 cases. The American Journal of Gastroenterology, 80(10), 774-778.

Edelman, A. S., & Zolla-Pazner, S. (1989). AIDS: a syndrome of immune dysregulation, dysfunction, and deficiency. FASEB Journal, 3, 22-30.

Farshtchi, D., & Lewis, V. J. (1968). Effects of three bacterial infections on serum lipids of rabbits.

Journal of Bacteriology, 95(5), 1615-1621.

Fauci, A. S. (1988). The human immunodeficiency virus: infectivity and mechanisms of pathogenesis. Science, 239, 617-622.

Feigin, R. D., & Dangerfield, H. G. (1967). Whole blood amino acid changes following respiratory-acquired Pasteurella tularensis infection in man. Journal of Infectious Disease, 117, 346-351.

Feigin, R. D., Klainer, A. S., Beisel, W. R., & Hornick, R. B. (1968). Whole-blood amino acids in experimentally induced typhoid fever in man. The New England Journal of Medicine, 278(6), 293-298.

Feingold, K. R. & Grunfeld, C. (1987). Tumor necrosis factor-alpha stimulates hepatic lipogenesis in the rat in vivo. Journal of Clinical Investigation, 80, 184-190.

Feingold, K. R., Soued, M., Staprans, I., Gavin, L. A., Donahue, M. E., Huang, B.-J., Moser, A. H., Gulli, R., & Grunfeld, C. (1989). Effect of tumor necrosis factor (TNF) on lipid metabolism in the diabetic rat: Evidence that inhibition of adipose tissue lipoprotein lipase activity is not required for TNF-induced hyperlipidemia. Journal of Clinical Investigation, 83, 1116-1121.

Fiser, R. H., Denniston, J. C., & Beisel, W. R. (1972). Infection with Diplococcus pneumoniae and Salmonella

typhimurium in monkeys: Changes in plasma lipids and lipoproteins. The Journal of Infectious Diseases, 125(1), 54-60.

Flores, E. A., Bistrian, B. R., Pomposelli, J. J., Dinarello, C. A., Blackburn, G. L., & Istfan, N. W. (1989). Infusion of tumor necrosis factor/ cachectin promotes muscle catabolism in the rat: A synergistic effect with interleukin-1. Journal of Clinical Investigation, 83, 1614-1622.

Fong, Y., Moldawer, L. L., Marano, M., Wei, H., Barber, A., Manogue, K., Tracey, K. J., Kuo. G., Fischman, D. A., Cerami, A., & Lowry, S. F. ((1989). Cachectin/ TNF or IL-1 alpha induces cachexia with redistribution of body proteins. American Journal of Physiology, 256, R659-R665.

Fox, C. H., Kotler, D., Tierney, A., Wilson, C. S., & Fauci, A. S. (1989). Detection of HIV-1 RNA in the Lamina Propria of Patients with AIDS and Gastrointestinal Disease. The Journal of Infectious Diseases, 159(3), 467-471.

Frei, L., & Steigbigel, R. (1988). Severe malnutrition in a young man with AIDS. Nutrition Reviews, 46(3), 126-132.

Gallin, J. I., Kaye, D., & O'Leary, W. M. (1969). Serum lipids in infection. The New England Journal of Medicine, 281(20), 1081-1086.

Gallin, J. I., O'Leary, W. M., & Kaye, D. (1970). Serum

concentrations of lipids in rabbits infected with Escherichia coli and Staphylococcus aureus. Proceedings of the Society of Experimental Biology and Medicine, 133, 309-313.

Garcia, M. E., Collins, C. L., & Mansell, W. A. (1987). The acquired immune deficiency syndrome: Nutritional complications and assessment of body weight status. Nutrition in Clinical Practice, 2, 108-111.

Gelmann, E. P., & Broder, S. (1987). Kaposi's Sarcoma in the setting of the AIDS pandemic. In S. Broder (Ed.), AIDS: Modern concepts and therapeutic challenges (pp. 219-232). New York: Marcel Dekker.

George, D. T., Rayfield, E. J., & Wannemacher, R. W., Jr. (1974). Altered glucoregulatory hormones during acute pneumococcal sepsis in the rhesus monkey. Diabetes, 23(6), 544-549.

Gillin, J. S., Shike, M., Alcock, N., Urmacher, C., Krown, S., Kurtz, R. C., Lightdale, C. J., & Winawer, S. J. (1985). Malabsorption and mucosal abnormalities of the small intestine in the Acquired Immunodeficiency Syndrome. Annals of Internal Medicine, 102, 619-622.

Girardin, E., Grau, G. E., Dayer, J., Roux-Lombard, P., The J5 Study Group, & Lambert, P. (1988). Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. The New England Journal of Medicine, 319(7), 397-400.

Glasgow, B. J., Anders, K., Layfield, L. J., Steinsapir, K. D., Gitnick, G. L., & Lewin, K. J. (1985). Clinical and pathologic findings of the liver in the Acquired Immune Deficiency Syndrome (AIDS). American Journal of Clinical Pathology, 83, 582-588.

Goldberg, A. L., & Chang, T. W. (1978). Regulation and significance of amino acid metabolism in skeletal muscle. Federation Proceedings, 37, 2301-2307.

Golden, M. H. N., & Waterlow, J. C. (1977). The in vivo measurement of protein synthesis. The American Journal of Clinical Nutrition, 30, 1353-1354.

Goldstein, S. A., & Elwyn, D. H. (1989). The effects of injury and sepsis on fuel utilization. Annual Reviews of Nutrition, 9, 445-473.

Gordin, F. M., Simon, G. L., Wofsy, C. B., & Mills, J. (1984). Adverse reactions to trimethoprim-sulfamethoxazole in patients with the acquired immunodeficiency syndrome. Annals of Internal Medicine, 100, 495-499.

Grau, G. E., Taylor, T. E., Molyneux, M. E., Wirima, J. J., Vassalli, P., Hommel, M., & Lambert, P.-H. (1989). Tumor necrosis factor and disease severity in children with falciparum malaria. The New England Journal of Medicine, 320, 1586-1591.

Gray, R. H. (1983). Similarities between AIDS and PCM. American Journal of Public Health, 73(11), 1332.

Groopman, J. E. (1986). Clinical symptomatology of the Acquired Immunodeficiency Syndrome (AIDS) and related disorders. Progress in Allergy, 37, 182-193.

Groopman, J. E. (1987a). Neoplasms in the Acquired Immune Deficiency Syndrome: the multidisciplinary approach to treatment. Seminars in Oncology, 14(2),Suppl 3, 1-6.

Groopman, J. E. (1987b). Spectrum of HTLV-III infection. In S. Broder (Ed.), AIDS: Modern concepts and therapeutic challenges (pp. 135-142). New York: Marcel Dekker.

Grunfeld, C., Kotler, D. P., Hamadeh, R., Tierney, A., Wang, J., & Pierson, R. N., Jr. (1989). Hypertriglyceridemia in the Acquired Immunodeficiency Syndrome. The American Journal of Medicine, 86, 27-31.

Grunfeld, C., Wilking, H., Neese, R., Gavin, L. A., Moser, A. H., Gulli, R., Serio, M. K., & Feingold, K. R. (1989). Persistence of the hypertriglyceridemic effect of tumor necrosis factor despite development of tachyphylaxis to its anorectic/ cachectic effects in rats. Cancer Research, 49, 2554-2560.

Hartiala, K. (1973). Metabolism of hormones, drugs and other substances by the gut. Physiological Reviews, 53(2), 496-534.

Hellerstein, M. K. (1986). Glycoconjugates as non-invasive probes of intrahepatic metabolism. Ph.D. Dissertation, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Hellerstein, M. K. (1989). In vivo measurement of hepatic UDP-glucose appearance rate and partitioning of gluconeogenic flux in fasted and refed rats. Unpublished manuscript.

Hellerstein, M. K. (1988a, December). New non-invasive stable isotopic probes for studying regulation of hepatic metabolic pathways in man. Paper presented at the American Diabetes Association Annual Research Symposium, San Francisco, CA.

Hellerstein, M. K. (1988b). Substrate pathways, monokines, wasting, and HIV infection. Grant submitted to and funded by NIH.

Hellerstein, M. K., Greenblatt, D. J., & Munro, H. N. (1986). Glycoconjugates as noninvasive probes of intrahepaic metabolism: Pathways of glucose entry into compartmentalized hepatic UDP-glucose pools during glycogen accumulation. Proceedings of the National Academy of Sciences, U.S.A., 83, 7044-7048.

Hellerstein, M. K., Greenblatt, D. J., & Munro, H. N. (1987). Glycoconjugates as noninvasive probes of intrahepatic metabolism: I. Kinetics of label incorporation with evidence of a common precursor UDP-glucose pool for secreted glycoconjugates. Metabolism, 36(10), 988-994.

Hellerstein, M. K., & Munro, H. N. (1987). Glycoconjugates as noninvasive probes of intrahepatic metabolism: II.

Application to measurement of plasma alpha 1-acid glycoprotein turnover during inflammation. Metabolism, 36(10), 995-1000.

Hellerstein, M. K., & Munro, H. N. (1988). Glycoconjugates as noninvasive probes of intrahepatic metabolism: III. Application to galactose assimilation by the intact rat. Metabolism, 37(4), 312-317.

Hellerstein, M. K., Wu, K., Kaempfer, S., Lee, W. P., Reid, S., & Shackleton, C. H. L. (1989). Non-invasive studies of intracellular metabolism in human subjects using mass spectrometry (MS). I) Glucuronide probe. FASEB Journal, 3, A243.

Hellerstein, M. K., Xie, Y., & Munro, H. N. (1987). In vivo assessment of pyruvate dehydrogenase activity and its role in hepatic UDP-glucose and glycogen synthesis in the rat. Clinical Research, 35(3), 506A.

Hetenyi, G., Jr. (1982). Correction for the metabolic exchange of 14C for 12C atoms in the pathway of gluconeogenesis in vivo. Federation Proceedings, 41, 104-109.

Hetenyi, G., Jr., Perez, G., & Vranic, M. (1983). Turnover and precursor-product relationships of nonlipid metabolites. Physiological Reviews, 63(2), 606-667.

Hirsch, R. L., McKay, D. G., Travers, R. I., & Skraly, R. K. (1964). Hyperlipidemia, fatty liver, and bromsulfophthalein retention in rabbits injected

intravenously with bacterial endotoxins. Journal of Lipid Research, 5, 563-568.

Ho, D. D., Pomerantz, R. J., & Kaplan, J. C. (1987). Pathogenesis of infection with human immunodeficiency virus. The New England Journal of Medicine, 317(5), 278-286.

Holness, M. J., French, T. J., & Sugden, M. C. (1986). Hepatic glycogen synthesis on carbohydrate re-feeding after starvation: a regulatory role for pyruvate dehydrogenase in liver and extrahepatic tissues. Biochemical Journal, 235, 441-445.

Holness, M. J. & Sugden, M. C. (1989). Pyruvate dehydrogenase activities during the fed-to-starved transition and on re-feeding after acute or prolonged starvation. Biochemical Journal, 258, 529-533.

Hopefl, A. W. (1988). What is the role of parenteral nutrition in AIDS? Clinical Pharmacy, 7, 512-513.

Janson, D. D., & Teasley, K. M. (1988). Parenteral nutrition in the management of gastrointesitnal Kaposi's sarcoma in a patient with AIDS. Clinical Pharmacy, 7, 536-544.

Kaempfer, S., Wu., K., & Hellerstein, M. K. (1989). Non-invasive studies of intrahepatic metabolism in rats using the acetyl probe. FASEB Journal, 3, A244.

Kaminski, M. V., Jr., Neufeld, H. A., & Pace, J. G. (1979). Effect of inflammatory and noninflammatory stress on plasma ketone bodies and free fatty acids and on

glucagon and insulin in peripheral and portal blood. Inflammation, 3(3), 289-294.

Katz, J. (1985). Determination of gluconeogenesis in vivo with 14C-labeled substrates. American Journal of Physiology, 17, R391-R399.

Katz, J., & McGarry, J. D. (1984). The glucose paradox: Is glucose a substrate for liver metabolism? Journal of Clinical Investigation, 74, 1901-1909.

Katz, J., & Rognstad, R. R. (1976). Futile cycles in the metabolism of glucose. In E. R. Stadtman & B. Horecker, (Eds.), Current Topics in Cellular Regulation, Vol. 10, (pp. 237-289). New York: Academic Press.

Kaufmann, R. L., Matson, C. F., Rowberg, A. H., & Beisel, W. R. (1976).Defective lipid disposal mechanisms during bacterial infection in rhesus monkeys. Metabolism, 25(6), 615-624.

Kawakami, M., Pekala, P. H., Lane, M. D., & Cerami, A. (1982). Lipoprotein lipase suppression in 3T3-L1 cells by an endotoxin-induces mediator from exudate cells. Proceedings of the National Academy of Sciences, U.S.A., 79, 912-916.

Keithley, J. K. & Kohn, C. L. (1990). Managing nutritional problems in people with AIDS. Oncology Nursing Forum, 17(1), 23-27.

Kern, P., Hemmer, C.-F., Van Damme, J., Gruss, H.-J., & Dietrich, M. (1989). Elevated tumor necrosis factor

alpha and interleukin-6 serum levels as markers for complicated Plasmodium falciparum malaria. The American Journal of Medicine, 87, 139-143.

Kerttula, Y., & Weber, T. H. (1986). Serum lipids in viral and bacterial meningitis. Scandinavian Journal of Infectious Disease, 18, 211-215.

Kotler, D. P. (1987). Why study nutrition in AIDS? Nutrition in Clinical Practice, 2, 94-95.

Kotler, D. P. (1989). Intestinal and hepatic manifestations of AIDS. Advances in Internal Medicine, 34, 43-72.

Kotler, D. P., Gaetz, H. P., Lange, M., Klein, E. B., & Holt, P. R. (1984). Enteropathy associated with the Acquired Immunodeficiency Syndrome. Annals of Internal Medicine, 101(4), 421-428.

Kotler, D. P., Scholes, J. V., & Tierney, A. R. (1987). Intestinal plasma cell alterations in Acquired Immunodeficiency Syndrome. Digestive Diseases and Sciences, 32(2), 129-138.

Kotler, D. P., Tierney, A. R., Altilio, D., Wang, J., & Pierson, R. N. (1989). Body mass repletion during Ganciclovir treatment of cytomegalovirus infections in patients with acquired immunodeficiency syndrome. Archives of Internal Medicine, 149, 901-905.

Kotler, D. P., Tierney, A. R., Brenner, S. K., Couture, S., Wang, J., & Pierson, R. N., Jr. (1990). Preservation of short-term energy balance in clinically stable patients

with AIDS. American Journal of Clinical Nutrition, 51, 7-13.

Kotler, D. P., Tierney, A. R., Wang, J., & Pierson, R. N., Jr. (1989). Magnitude of body-cell-mass depletion and the timing of death from wasting in AIDS. American Journal of Clinical Nutrition, 50, 444-447.

Kotler, D. P., Wang, J., & Pierson, R. N. (1985). Body composition studies in patients with the acquired immunodeficiency syndrome. The American Journal of Clinical Nutrition, 42, 1255-1265.

Kovacs, J. A., & Masur, H. (1988). Opportunistic infections. In V. T. DeVita, Jr., S. Hellman, & S. A. Rosenberg (Eds.), AIDS: Etiology, diagnosis, treatment, and prevention (2nd Edition)(pp. 199-225) Philadelphia: J. B. Lippincott.

Krebs, H. A., Hems, R., Weidemann, M. J., & Speake, R. N. (1966). Biochemical Journal, 101, 242-249.

Kushner, I. (1982). The phenomenon of the acute phase response. Annals of the New York Academy of Sciences, 389, 39-48.

Lahdevirta, J., Maury, C. P. J., Teppo, A.-M., & Repo, H. (1988). Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. The American Journal of Medicine, 85, 289-291.

Lang, C. H., Bagby, G. J., Blakesley, H. L., Johnson, J. L.,

& Spitzer, J. J. (1986). Plasma glucose concentration determines direct versus indirect liver glycogen synthesis. American Journal of Physiology, 251, E584-E590.

LaNoue, K. F., Mason, A. D., Jr., & Daniels, J. P. (1968). The impairment of glucogenesis by gram negative infection. Metabolism, 17(7), 606-611.

Laskin, O. L., de Miranda, P., & Blum, M. R. (1989). Azidothymidine steady-state pharmacokinetics in patients with AIDS and AIDS-related complex. The Journal of Infectious Diseases, 159(4), 745-747.

Lau, A. S., & Livesey, J. F. (1989). Endotoxin induction of tumor necrosis factor is enhanced by acid-labile interferon-alpha in Acquired Immunodeficiency Syndrome. Journal of Clinical Investigation, 84, 738-743.

Le, J., & Vilcek, J. (1987). Tumor necrosis factor and interleukin-1: cytokines with multiple overlapping biological activities. Laboratory Investigation, 56(3), 234-248.

Lebovics, E., & Dworkin, B. M. (1987). The liver in AIDS. In G. P. Wormser, R. E. Stahl, & E. J. Bottone (Eds.) Acquired Immune Deficiency Syndrome and other manifestations of HIV infection (pp. 767-782). Park Ridge, New Jersey: Noyes Publications.

Lebovics, E., Thung, S. N., Schaffner, F., & Radensky, P. W. (1985). The liver in the Acquired Immunodeficiency

Syndrome: a clinical and histologic study. Hepatology, 5(2), 293-298.

Lees, R. S., Fiser, R. H., Jr., Beisel, W. R., & Bartelloni, P. J. (1972). Effects of an experimental viral infection on plasma lipid and lipoprotein metabolism. Metabolism, 21(9), 825-833.

Liao, Z., & Rosenstreich, D. L. (1983). An interleukin-1 inhibitor in the urine of febrile patients. Clinical Research, 31(2), 492A.

Lundquist, F. (1962). Production and utilization of free acetate in man. Nature, 193(4815), 579-580.

Lust, G. (1966). Effect of infection on protein and nucleic acid synthesis in mammalian organs and tissues. Federation Proceedings, 25, 1688-1694.

Mahoney, J. R., Jr., Beutler, B. A., LeTrang, N., Vine, W., Ikeda, Y., Kawakami, M., & Cerami, A. (1985). Lipopolysaccaride-treated RAW 264.7 cells produce a mediator that inhibits lipoprotein lipase in 3T3-L1 cells. Journal of Immunology, 134(3), 1673-1675.

McGarry, J. D., & Foster, D. W. (1980). Regulation of hepatic fatty acid oxidation and ketone body production. Annual Review of Biochemistry, 49, 395-420.

McGarry, J. D., Kuwajima, M., Newgard, C. B., & Foster, D. W. (1987). From dietary glucose to liver glycogen: the full circle round. Annual Reviews of Nutrition, 7, 51-73.

Michie, H. R., Manogue, K. R., Spriggs, D. R., Revhaug, A., O'Dwyer, S., Dinarello, C. A., Cerami, A., Wolff, S. M., & Wilmore, D. W. (1988). Detection of circulating tumor necrosis factor after endotoxin administration. The New England Journal of Medicine, 318(23), 1481-1486.

Mizel, S. B. (1989). The interleukins. FASEB Journal, 3, 2379-2388.

Molina, J.-M., Scadden, D. T., Byrn, R., Dinarello, C. A., & Groopman, J. E. (1989). Production of tumor necrosis factor alpha and interleukin-1 beta by monocytic cells infected with human immunodeficiency virus. Journal of Clinical Investigation, 84, 733-737.

Moore, R. N., Johnson, B. A., & Berry, L. J. (1977). Nutritional effects of salmonellosis in mice. The American Journal of Clinical Nutrition, 30, 1289-1293.

Morland, J. & Olsen, H. (1977). Metabolism of sulfadimidine, sulfanilamide, p-aminobenzoic acid, and isoniazid in suspensions of parenchymal and nonparenchymal rat liver cell. Drug Metabolism and Disposition, 5(6), 511-517.

Munro, H. N. (1974). Protein metabolism in response to injury and other pathological conditions. Acta Anaesthesiologia Scandinavica, Suppl. 55, 81-86.

Nakanuma, Y., Liew, C. T., Peters, R. L., & Govindarajan, S. (1986). Pathologic features of the liver in acquired immune deficiency syndrome (AIDS). Liver, 6, 158-166.

Nawabi, M. D., Block, K. P., Chakrabarti, M. C., & Buse, M. G. (1990). Administration of endotoxin, tumor necrosis factor, or interleukin 1 to rats activates skeletal muscle branched-chain alpha-keto acid dehydrogenase. Journal of Clinical Investigation, 85, 256-263.

Nelson, J. A., Reynolds-Kohler, C., Margaretten, W., Wiley, C. A., Reese, C. E., & Levy, J. A. (1988). Human immunodeficiency virus detected in bowel epithelium from patients with gastrointestinal symptoms. The Lancet, 1, 259-262.

Neufeld, H. A., Kaminski, M. V., Jr., & Wannemacher, R. W., Jr. (1977). Effect of inflammatory and noninflammatory stress on ketone bodies and free fatty acids in rats. American Journal of Clinical Nutrition, 30, 1357-1358.

Neufeld, H. A., Pace, J. G., Kaminski, M. V., George, D. T., Jahrling, P. B., Wannemacher, R. W., Jr., & Beisel, W. R. (1980). A probable endocrine basis for the depression of ketone bodies during infectious or inflammatory states in rats. Endocrinology, 107(2), 596-601.

Neufeld, H. A., Pace, J. A., & White, F. E. (1976). The effect of bacterial infections on ketone concentrations in rat liver and blood and on free fatty acid concentrations in rat blood. Metabolism, 25(8), 877-884.

Newsholme, E. A., & Leech, A. R. (1983). Biochemistry for

the Medical Sciences. (pp. 336-356; 536-549) New York: John Wiley.

Newsholme, E. A., & Start, C. (1973). Regulation in Metabolism. (pp. 247-323). New York: John Wiley & Sons.

Olsen, H. (1982). Interaction between drug acetylation and ethanol, acetate, pyruvate, citrate, and L(-)carnitine in isolated rat liver parenchymal cells. Acta Pharmacol et Toxicol, 50, 67-74.

Olsen, H. & Morland, J. (1978). Ethanol-induced increase in drug acetylation in man and isolated rat liver cells. British Medical Journal, 2, 1260-1262.

Olsen, H. & Morland, J. (1983). Ethanol interaction with drug acetylation in vivo and in vitro. Pharmacology, Biochemistry & Behavior, 18(Suppl. 1), 295-300.

O'Sullivan, P., Linke, R. A., & Dalton, S. (1985). Evaluation of body weight and nutritinal status among AIDS patients. Journal of the American Dietetic Association, 85(11), 1483-1484.

Pace, J. G., Beall, F. A., Foulke, M. D., Neufeld, H. A., & Wannemacher, R. W., Jr. (1978). Regulation of fatty acid utilization in isolated perfused livers from Streptococcus pneumoniae infected rats. Clinical Research, 26, 627A.

Pace, J. A., Beall, F. A., Neufeld, H. A., & Wannemacher, R. W., Hr. (1977). Alterations in carnitine acylation states in S. pneumoniae infected rats. Federation

Proceedings, 36, 788.

Paretsky, D., Downs, C. M., & Salmon, C. W. (1964). Some biochemical changes in the guinea pig during infection with Coxiella Burnetii, Journal of Bacteriology, 88(1), 137-142.

Parrilla, R., Goodman, M. N., & Toews, C. J. (1974). Effect of glucagon: insulin ratios on hepatic metabolism. Diabetes, 23(9), 725-731.

Powanda, M. C. (1977). Changes in body balances of nitrogen and other key nutrients: description and underlying mechanisms. The American Journal of Clinical Nutrition, 30, 1254-1268.

Powanda, M. C., Dinterman, R. E., Wannemacher, R. W., Jr., & Herbrandson, G. D. (1974). Distribution and metabolism of phenylalanine and tyrosine during tularaemia in the rat. Biochemical Journal, 144, 173-176.

Powanda, M. C., Wannemacher, R. W., Jr., & Cockerell, G. L. (1972). Nitrogen metabolism and protein synthesis during pneumococcal sepsis in rats. Infection and Immunity, 6(3), 266-271.

Randle, P. J., Garland, P. B., Hales, C. N., & Newsholme, E. A. (1963). The glucose fatty-acid cycle: Its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. The Lancet, i, 785-789.

Rapoport, M. I., Lust, G., & Beisel, W. R. (1968). Host enzyme induction of bacterial infection. Archives of

Internal Medicine, 121, 11-16.

Rayfield, E. J., Curnow, R. T., George, D. T., & Beisel, W. R. (1973). Impaired carbohycrate metabolism during a mild viral illness. The New England Journal of Medicine, 289(12), 618-621.

Rayfield, E. J., Curnow, R. T., Reinhard, D., & Kochicheril, N. M. (1977). Effects of acute endotoxemia on glucoregulation in normal and diabetic subjects. Journal of Clinical Endocrinology and Metabolism, 45, 513-521.

Reddy, M. M., Sorrell, S. J., Lange, M., & Grieco, M. H. (1988). Tumor necrosis factor and HIV P24 antigen levels in serum of HIV-infected populations. Journal of Acquired Immune Deficiency Syndromes, 1, 436-440.

Reeve, E. B., Pearson, J. R., & Martz, D. C. (1963). Plasma protein synthesis in the liver. Method for measurement of albumin formation in vivo. Science, 139, 914-916.

Resler, S. S. (1988). Nutrition care of AIDS patients. Journal of the American Dietetic Association, 88, 828-832.

Resnick, L., & Herbst, J. S. (1987). Dermatological (Non-Kaposi's Sarcoma) manifestations associated with HTLV-III/LAV infection. In S. Broder (Ed.), AIDS: Modern concepts and therapeutic challenges (pp. 285-302). New York: Marcel Dekker.

Richman, D. D., Fischl, M. A., Grieco, M. H., Gottlieb, M.

S., Volberding, P. A., Laskin, O. L., Leedom, J. M., Groopman, J. E., Mildvan, D., Hirsch, M. S., Jackson, G. G., Durack, D. T., Nusinoff-Lehrman, S., & the AZT Collaborative Working Group. (1987). The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex: A double-blind, placebo-controlled trial. The New England Journal of Medicine, 317, 192-197.

Rocha, D. M., Santeusanio, F., Faloona, G. R., & Unger, R. H. (1973). Abnormal pancreatic alpha-cell function in bacterial infections. The New England Journal of Medicine, 288(14), 700-703.

Ruderman, N. B. (1975). Muscle amino acid metabolism and gluconeogenesis. Annual Review of Medicine, 26, 245-258.

Ryan, N. T. (1976). Metabolic adaptations for energy production during trauma and sepsis. Surgical Clinics of North America, 56(5), 1073-1090.

Safai, B. (1987). Kaposi's Sarcoma: An overview of classical and epidemic forms. In S. Broder (Ed.), AIDS: Modern concepts and therapeutic challenges (pp. 205-218). New York: Marcel Dekker.

Sammalkorpi, K., Valtonen, V., Kerttula, Y., Nikkila, E., & Taskinen, M. (1988). Changes in serum lipoprotein pattern induced by acute infections. Metabolism, 37(9), 859-865.

Sato, P. A., Chin, J., & Mann, J. M. (1989). Review of AIDS and HIV infection: global epidemiology and statistics. AIDS, 3(Suppl), S301-S307.

Saudek, C. D., & Felig, P. (1976). The metabolic events of starvation. The American Journal of Medicine, 60, 117-126.

Seligmann, M., Pinching, A. J., Rosen, F. S., Fahey, J. L., Khaitov, R. M., Klatzmann, D., Koenig, S., Luo, N., Ngu, J., Riethmuller, G., & Spira, T. J. (1987). Immunology of human immunodeficiency virus infection and the Acquired Immunodeficiency Syndrome. An update. Annals of Internal Medicine, 107, 234-242.

Semb, H., Peterson, J., Tavernier, J., & Olivecrona, T. (1987). Multiple effects of tumor necrosis factor on lipoprotein lipase in vivo. The Journal of Biological Chemistry, 262(17), 8390-8394.

Shafer, R. W., Seitzman, & Tapper, M. L. (1989). Successful prophylaxis of Pneumocystis carinii pneumonia with Trimethoprim- Sulfamethoxazole in AIDS patients with previous allergic reactions. Journal of Acquired Immune Deficiency Syndromes, 2, 389-393.

Shambaugh, G. E., & Beisel, W. R. (1967). Insulin response during tularemia in man. Diabetes, 16(6), 369-376.

Sigel, C. W. (1983). Disposition and metabolism of trimethoprim, tetroxoprim, sulfamethoxazole, and sulfadiazine. In G. H. Hitchings (Ed.) Inhibition of

folate metabolism in chemotherapy: The origins and uses of co-trimoxazole, (pp. 163-184). New York: Springer-Verlag.

Singh, V. N., Venkitasubramanian, T. A., & Viswanathan, R. (1963). Study of glucose tolerance and synthesis of hepatic glycogen from glucose and glycine-1-14C in tuberculous guinea pigs. Archives of Biochemistry and Biophysics, 101, 229-233.

Singh, V. N., Bhargava, U., Venkitasubramanian, T. A., & Viswanathan, R. (1963). Study of glycogen synthesizing and degrading enzymes of guinea pig liver in experimental tuberculosis. Archives of Biochemistry and Biophysics, 101, 234-239.

Snyder, I. S., Deters, M., & Ingle, J. (1971). Effect of endotoxin on pyruvate kinase activity in mouse liver. Infection and Immunity, 4(2), 138-142.

Strisower, E. H., Kohler, G. D., & Chaikoff, I. L. (1952). Incorporation of acetate carbon into glucose by liver slices from normal and alloxan-diabetic rats. Journal of Biological Chemistry, 198, 115-126.

Swick, R. W. (1958). Measurement of protein turnover in rat liver. Journal of Biological Chemistry, 228, 751-764.

Task Force on Nutrition Support in AIDS. (1989). Guidelines for Nutrition Support in AIDS. Nutrition, 5(1), 39-46.

Tchekmedyian, N. S., Tait, N., Moody, M., & Aisner, J. (1987). High-dose megestrol acetate: a possible

treatment for cachexia. Journal of the American Medical Association, 257, 1195-1198.

Ullrich, R., Zeitz, M., Heise, W., L'age, M., Hoffken, G., & Riecken, E. O. (1989). Small intestinal structure and function in patients infected with human immunodeficiency virus (HIV): Evidence for HIV-induced enteropathy. Annals of Internal Medicine, 111, 15-21.

Unger, R. H. (1971). Glucagon and the insulin: glucagon ratio in diabetes and other catabolic illnesses. Diabetes, 20(12), 834-838.

Vary, T. C. & Murphy, J. M. (1989). Role of extra-splanchnic organs in the metabolic response to sepsis: effect of insulin. Circulatory Shock, 29, 41-57.

Vary, T. C., Siegel, J. H., Nakatani, T., Sato, T., & Aoyama, H. (1986a). A biochemical basis for depressed ketogenesis in sepsis. The Journal of Trauma, 26(5), 419-425.

Vary, T. C., Siegel, J. H., Nakatani, T., Sato, T., & Aoyama, H. (1986b). Effect of sepsis on activity of pyruvate dehydrogenase complex in skeletal muscle and liver. American Journal of Physiology, 250, E634-E640.

Vary, T. C., Siegel, J. H., Tall, B. D., Morris, J. G., & Smith, J. A. (1988). Inhibition of skeletal muscle protein synthesis in septic intra-abdominal abscess. The Journal of Trauma, 28(7), 981-988.

Vary, T. C., Siegel, J. H., Zechnich, A., Tall, B. D.,

Morris, J. G., Placko, R., & Jawor, D. (1988). Pharmacological reversal of abnormal glucose regulation, BCAA utilization, and muscle catabolism in sepsis by dichloroacetate. The Journal of Trauma, 28(9), 1301-1311.

Viteri, F. E. (1981). Primary protein-energy malnutrition: Clinical, biochemical, and metabolic changes. In R. M. Suskind (Ed.), Textbook of Pediatric Nutrition, (pp. 189-215). New York: Raven.

Volberding, P. (1986). Introduction. In P. Jones (Ed.) Proceedings of the AIDS conference 1986, Newcastle upon Tyne, UK (pp. 7-21) Ponteland, Newcastle upon Tyne: Intercept.

Von Roenn, J. H., Murphy, R. L., Weber, K. M., Williams, L. M., & Weitzman, S. A. (1988). Megestrol acetate for treatment of cachexia associated with human immunodeficiency virus (HIV) infection. Annals of Internal Medicine, 109(10), 840-841.

Weber, A. & Opheim, K. E. (1983). High-performance liquid chromatographic quantitation of trimethoprim, sulfamethoxazole, and N4-acetylsulfamethoxazole in body fluids. Journal of Chromatography, 278, 337-345.

Wannemacher, R. W., Jr. (1977). Key role of various individual amino acids in host response to infection. The American Journal of Clinical Nutrition, 30, 1269-1280.

Wannemacher, R. W., Jr., Dinterman, R. E., Pekarek, R. S., Bartelloni, P. J., & Beisel, W. R. (1975). Urinary amino acid excretion during experimentally induced sandfly fever in man. The American Journal of Clinical Nutrition, 28, 110-118.

Wannemacher, R. W., Jr., Klainer, A. S., Dinterman, R. E., Beisel, W. R. (1976). The significance and mechanism of an increased serum phenylalanine-tyrosine ratio during infection. The American Journal of Clinical Nutrition, 29, 997-1006.

Wannemacher, R. W., Jr., Neufeld, H. A., & Canonico, P. G. (1976). Hepatic gluconeogenic capacity and rate during pneumococcal infection in rats. Federation Proceedings, 35, 343.

Wannemacher, R. W., Jr., Pace, J. G., Beall, F. A., Dinterman, R. E., Petrella, V. J., & Neufeld, H. A. (1979). Role of the liver in regulatin of ketone body production during sepsis. The Journal of Clinical Investigation, 64, 1565-1572.

Wannemacher, R. W., Jr., Pekarek, R. S., Bartelloni, P. J., Vollmer, R. T., & Beisel, W. R. (1972). Changes in individual plasma amino acids following experimentally induced sandfly fever virus infection. Metabolism, 21(1), 67-76.

Wannemacher, R. W., Jr., Powanda, M. C., Pekarek, R. S., & Beisel, W. R. (1971). Tissue amino acid flux after

exposure of rats to Diplococcus pneumoniae. Infection and Immunity, 4(5), 556-562.

Waterlow, J. C., Garlick, P. J., & Millward, D. J. (1978). Protein turnover in mammalian tissues and in the whole body. (pp. 179-223). New York: North-Holland.

Weinman, E. O., Strisower, E. H., & Chaikoff, I. L. (1957). Conversion of fatty acids to carbohydrate: Application of isotopes to this problem and role of the Krebs cycle as a synthetic pathway. Physiological Reviews, 37, 252-272.

Weiss, L., Haeffner-Cavaillon, N., Laude, M., Gilquin, J., & Kazatchkine, M. D. (1989). HIV infection is associated with the spontaneous production of interleukin-1 (IL-1) in vivo and with an abnormal release of IL-1alpha in vitro. AIDS, 3, 695-699.

Wharton, J. M., Coleman, D. L., Wofsy, C. B., Luce, J. M., Blumenfeld, W., Hadley, W. K., Ingram-Drake, L., Volberding, P. A., & Hopwell, P. C. (1986). Trimethoprim-sulfamethoxazole or pentamidine for Pneumocystis carinii pneumonia in the Acquired Immunodeficiency Syndrome. A prospective randomized trial. Annals of Internal Medicine, 105, 37-44.

Williams, C. A. (1965). Serum protein synthesis in normal and staphylococcus infected mice. Federation Proceedings, 24, 506.

Wilmore, D. W. (1976). Hormonal responses and their effect

on metabolism. Surgical Clinics of North America, 56(5), 999-1018.

Wolfe, R. R. (1984). Tracers in metabolic research: Radioisotope and stable isotope/ mass spectrometry methods. (pp. 1-26). New York: Alan R. Liss.

Woodward, J. M., & Miraglia, G. J. (1964). Distribution of serum proteins in tularemic rats. Canadian Journal of Microbiology, 10, 243-247.

Wu, K., Kaempfer, S., Reid, S., Shackleton, C. H. L., & Hellerstein, M. K. (1989). Non-invasive studies of intrahepatic metabolism in humans. II). Acetyl probe. FASEB Journal, 3, A243.

Yarchoan, R., & Broder, S. (1988). Pharmacologic treatment of HIV infection. In V. T. DeVita, Jr., S. Hellman, & S. A. Rosenberg (Eds.), AIDS: Etiology, diagnosis, treatment and prevention, (2nd edition)(pp. 282-283). Philadelphia: J. B. Lippincott.

Yarchoan, R., Mitsuya, H., & Broder, S. (1989). Clinical and basic advances in the antiretroviral therapy of human immunodeficiency virus infection. The American Journal of Medicine, 87, 191-200.

Yarchoan, R., Mitsuya, H., Myers, C. E., & Broder, S. (1989). Clinical pharmacology of 3' -azido- 2',3' -dideoxythymidine (zidovudine) and related dideoxynucleosides. The New England Journal of Medicine, 321(11), 726-738.

Yarchoan, R., & Pluda, J. M. (1988). Clinical aspects of infection with AIDS retrovirus: Acute HIV infection, persistent generalized lymphadenopathy, and AIDS-related complex. In V. T. DeVita, Jr., S. Hellman, & S. A. Rosenberg (Eds.), AIDS: Etiology, diagnosis, treatment, and prevention (2nd edition)(pp. 107-120). Philadelphia: J. B. Lippincott.

Zenser, T. V., DeRubertis, F. R., George, D. T., & Rayfield, E. J. (1974). Infection-induced hyperglucagonemia and altered hepatic response to glucagon in the rat. American Journal of Physiology, 227(6), 1299-1305.

Zilversmit, D. B., Entenman, C., & Fishler, C. (1943). On the calculation of "turnover time" and "turnover rate" from experiments involving the use of labeling agents. Journal of General Physiology, 26, 325-331.

Appendix A

Consent Form #1

Healthy Volunteers

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
SAN FRANCISCO GENERAL HOSPITAL
INVESTIGATIONAL PROTOCOL

Consent to be a Research Subject

Study Title: Metabolic pathways, monokines and wasting in HIV infection.

A. Purpose and Background

Dr. Marc Hellerstein and his associates from the Department of Medicine at the University of California at San Francisco are conducting a study to investigate the metabolic causes of weight loss and appetite loss in Human Immunodeficiency Virus (HIV) infection. It is well recognized that many patients with HIV infection, with or without Acquired Immunodeficiency Syndrome (AIDS) lose weight with changes in body content of muscle, fat, vitamins, minerals, etc. The resulting malnutrition may cause further suppression of the immune system. For this reason, it is important to understand the causes of malnutrition in HIV infection. At present, however, the causes are not well understood. This study will attempt to determine whether metabolism of carbohydrates and fats is disordered in HIV infection, what hormones cause these abnormalities, and whether this could explain the weight loss and appetite loss. I have been asked to participate in this study because I am a normal volunteer with no history of weight loss or HIV infection.

B Procedures

If I agree to participate in this study, the following will happen:

1. I will be evaluated to see if I am eligible to be in this study. This includes: a history of my medical condition, a brief physical examination and some blood tests (two tablespoons of blood will be drawn).

2. If I am eligible to participate based on the above evaluation, I will come to the General Clinical Research Center (GCRC) of the San Francisco General Hospital one morning for pre-study testing. On this day, I will have my skinfold thickness determined by calipers, my diet history taken by a research dietitian, my body composition determined by impedance plethysmography and D20 ingestion, screening blood tests drawn, and a test dose of acetaminophen (Tylenol) and sulfamethoxazole (Gantanol)

given by mouth. Each of these will now be described:

a) Caliper measurements. This involves determining the thickness of my skinfolds to see how much fat I have at different areas of my body. It takes less than 15 minutes and is painless.

b) Dietary history. This involves careful questioning by the dietitian to determine how much I eat at home. This may take 30-60 minutes.

c) Impedance plethysmography. I will lie down on a cot and have electrodes attached to my arms. The resistance of my body to a small electrical impulse indicates how much body fat I have. This procedure is painless and takes 5-10 minutes.

d) D20 ingestion. This is a special "heavy" water, which can be easily measured. I will drink some as a small glass of water. It has no harmful effects and tastes like normal water. For the next five (5) hours, my urine will be collected.

e) Screening blood tests. Two tablespoons of blood will be drawn. Included in these tests will be a test for my HIV status. I will receive pre-test counseling on this day from counselors from the AIDS Health Project of UC San Francisco. These counselors are trained in pre- and post-test counseling of people getting HIV tested.

f) Test dose of acetaminophen (Tylenol) 650 mg and sulfamethoxazole (Gantanol) 500 mg. These will be given to me orally shortly after I arrive. My urine will be collected for the next 5 hours.

The visit on this day will last 5-6 hours and will be scheduled at my convenience.

3. I will than be scheduled to enter the GCRC for three (3) separate 24 hour (overnight) admissions, each at least a week apart. The three admissions will involve identical protocols on my part, except for the first admission when I will be informed by Dr. Hellerstein in person of my HIV test results. There will be post-test counseling available to me through the AIDS Health Project at no charge to me (see below).

4. I will enter the GCRC for each admission between 10:00 AM and Noon on the day scheduled. Lunch and supper

will be served and I can eat without any restrictions. After 8:00 PM, I cannot eat any food or drink calorie-containing beverages until the next morning's meal. I will tell the dietitian my choices for the next day's meal at this time also. From this point on, the following procedures will be performed:

a) Either at 8:00 PM or 8:00 AM the next morning, an intravenous line will be placed. This will stay in place until 6:00 PM the next day.

b) I will have a sample of my breath collected, which involves blowing into a small rubber tube and syringe apparatus for a few seconds.

c) Beginning during the night or the next morning, the nurse will begin infusions of special "heavy" sugars or others nutrients into the intravenous line. These contain no radioactivity nor is there any radioactivity involved at any time in this study. Each admission will involve infusions of different sugars or other nutrients (glucose, galactose, palmitate, acetate). These are given in "tracer" quantities, which means that the amount given is so small that it will not itself affect my metabolism. The intravenous solution will also contain acetaminophen (Tylenol), which will be given at 100 mg/hr. This is roughly the same dose of acetaminophen (Tylenol) that I might take orally for a cold or a head-ache. The infusion will continue until 6:00 PM.

d) I will also take one teaspoon (500 mg) of the antibiotic sulfamethoxazole (Gantanol) starting at midnight for 4 total doses (midnight, 4 AM, 8 AM, 1 PM) or starting at 10 AM for 3 total doses (10 AM, 1 PM, 4 PM). This is the approximate dose I would take of this medication if I had a urinary tract infection, bronchitis or other mild infection (sulfamethoxazole is present in the medications Bactrim and Septra, which are commonly used for various infections).

e) I will collect all my urine after midnight in the containers provided.

f) Between 7:30 and 9:00 AM, a second IV line will be placed for blood drawing, to avoid my having to get needle sticks every hour. Each hour from 8:00 or 9:00 AM, a sample of blood is drawn (less than 2 teaspoons) and a breath sample is collected, in addition to saving all my urine, until 6:00 PM. Twice in the morning (8:00 and 9:00 AM) and twice in the afternoon (3:00 and 4:00 PM), arterialized hand vein samples will be drawn. This consists of placing an intravenous butterfly line

in a vein in the back of my hand then placing the hand (wrapped in a plastic bag) in a luke-warm water bath for 5 minutes before collecting a blood sample.

g) Each hour from 8:00 AM, I will breath into a mask or tube for a few minutes to measure my oxygen use and carbon dioxide production.

h) I will be fed in the morning one of three ways--either with a breakfast, with Ensure (a liquid diet formula) or with intravenous sugar water (glucose). Breakfast or brunch will be served at 10:30 AM in accordance with my requests from the previous evening. I agree to try to eat the entire meal that is served. I can drink beverages of my choice during this meal, but they can contain no caffeine. Ensure feedings will begin either at 8:00 or 9:00 AM and continue hourly until 5:00 PM. There are several flavors which I may choose from (vanilla, strawberry, chocolate). Each hourly serving will be about 10 ounces. Intravenous sugar water (glucose) will begin at 8:00 AM and go until 6:00 PM. In my case, feeding will be with _____.

i) At 4:00 PM, I may receive an intravenous injection of glucagon (1 mg).

j) At 5:00 PM or 6:00 PM, the infusion will be discontinued and the first intravenous line removed. After the 6:00 PM samples are taken, the second intravenous line will be removed. The admission will be completed at this time (6:00 PM).

k) I may eat supper at this time in the GCRC and then leave or I may leave immediately at 6:00 PM.

C. Risks/Discomforts

1. Procedures. A hematoma (black and blue mark can result from placement of intravenous lines. This can be painful but carries no significant risks. Infections from intravenous lines rarely occur from such brief (24 hour) placement. However, any signs of inflammation (redness, tenderness, warmth of the skin over the catheter) will lead to removal of the catheter and treatment with warm soaks and elevation. The other procedures in these studies (breath and urine collections, diet) are not associated with any risks.

2. Acetaminophen administration. Acetaminophen (Tylenol) is given at 100 mg per hour for 15 hours or less. This is less than or similar to the usual oral dose taken in

over-the counter pills. The experience of the investigators with large numbers of volunteers receiving intravenous acetaminophen has revealed no problems or intolerance.

3. Sulfamethoxazole administration. If I have ever experienced an allergic reaction to a sulfa drug in the past, I will be excluded from this study. If I develop evidence of allergy during any study (rash, hives, shortness of breath) the drug will be halted immediately and treatment for the allergy will be given.

4. Stable isotope administration. The special "heavy" molecules I will receive contain no radioactivity and have no recognized adverse effects.

5. Glucagon administration. Glucagon is a natural hormone in my body, so there should be no possibility of my being allergic to it. I may feel slightly flushed or queasy briefly after glucagon is given, but no serious adverse effects are likely.

6. Blood drawing. The total amount of blood drawn over the 3 infusions will be 250 ml over the course of at least 3 weeks. This amount is well within the Red Cross' guidelines (less than 450 ml per month). If I have a low red blood cell count at any time during the study, I will be excluded from participating. I have been advised not to donate blood for at least 8 weeks following the completion of this study because of the blood drawing during this study.

7. Potential loss of privacy. Participation in research may involve loss of privacy. My research records will be handled as confidentially as is possible within the law. All records will be coded. No individual identities will be used in any reports or publications resulting from this study. While confidentiality cannot be guaranteed, it will be protected up to the full extent of the law.

8. Knowledge of HIV status. In order for me to enter this study as a normal control subject, HIV testing has to be performed on my blood. It is the policy of the University of California at San Francisco and the Federal Government (Public Health Service) that anyone in a study such as this who has HIV testing must be informed of the results and should receive pre- and post-test counseling. Dr. Hellerstein and his associates in this study agree with this policy. Therefore, if I choose to enter this study, I will be HIV tested and will receive pre-test counseling by counselors from the AIDS Health Project of the University of California at San Francisco. This counseling will occur at the San Francisco General Hospital on the day of pre-study testing. The counselors are trained in the counseling of

people getting HIV tested. I will be informed of the HIV test results by Dr. Hellerstein in person at the General Clinical Research Center during my first admission day. Post-test counseling will also be provided through the AIDS Health Project counselors.

Being tested for HIV can cause anxiety and stress. If I were to test positive, this would probably cause a great deal of stress and raise many concerns on my part. This is why I will receive pre- and post-test counseling from trained counselors.

D. Treatment and Compensation for injury

If I am injured as a result of being in this study, treatment will be available. The cost of such treatment may be covered by the University of California depending on a number of factors. The university does not normally provide any other form of compensation for injury. For further information, I may call the office of the Committee on Human Research at (415) 476-1814.

E. Benefits

I may benefit from participating in the study, by receiving nutritional education and information regarding my actual food intake. However, no benefit can be guaranteed to me.

F. Alternatives

The alternative is not to participate in the study.

G. Costs

There will be no charges to me from this study.

H. Reimbursement

I will receive $250 upon completion of the screening plus 3 GCRC admissions. I will receive a prorated portion of the $250 based on the percent of the study completed ($10 for the screening tests then $80 for each of the 3 admissions completed). If an extra admission is necessary, I will be paid $80 more.

I. Questions

This study has been explained to me by Dr. Hellerstein or _____ and my questions were answered. If I have any other questions about the study I may call Dr. Hellerstein at (415) 821-8982 or 642-0646.

J. Consent

I have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

Participation in research is voluntary. I have the right to decline to participate or to withdraw at any point in this study without jeopardy to my medical care.

If I wish to participate, I should sign below.

_____

Date

Subject's signature

_____

Person obtaining consent

APPENDIX B

Consent Form #2

Subjects with Asymptomatic HIV Infection

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
SAN FRANCISCO GENERAL HOSPITAL
INVESTIGATIONAL PROTOCOL

Consent to be a Research Subject

Study Title: Metabolic pathways, monokines and wasting in HIV infection.

A. Purpose and Background

Dr. Marc Hellerstein and his associates from the Department of Medicine at the University of California at San Francisco are conducting a study to investigate the metabolic causes of weight loss and appetite loss in Human Immunodeficiency Virus (HIV) infection. It is well recognized that many patients with HIV infection, with or without Acquired Immunodeficiency Syndrome (AIDS) lose weight with changes in body content of muscle, fat, vitamins, minerals, etc. The resulting malnutrition may cause further suppression of the immune system. For this reason, it is important to understand the causes of malnutrition in HIV infection. At present, however, the causes are not well understood. This study will attempt to determine whether metabolism of carbohydrates and fats is disordered in HIV infection, what hormones cause these abnormalities, and whether this could explain the weight loss and appetite loss. I have been asked to participate in this study because I have been diagnosed as having been infected with HIV but have not lost weight or had any other symptoms.

B Procedures

If I agree to participate in this study, the following will happen:

1. I will be evaluated to see if I am eligible to be in this study. This includes: a history of my medical condition, a brief physical examination and some blood tests (two tablespoons of blood will be drawn).

2. If I am eligible to participate based on the above evaluation, I will come to the General Clinical Research Center (GCRC) of the San Francisco General Hospital one morning for pre-study testing. On this day, I will have my skinfold thickness determined by calipers, my diet history taken by a research dietitian, my body composition determined by impedance plethysmography and D20 ingestion, screening blood tests drawn, and a test dose of

acetaminophen (Tylenol) and sulfamethoxazole (Gantanol) given by mouth. Each of these will now be described:

a) Caliper measurements. This involves determining the thickness of my skinfolds to see how much fat I have at different areas of my body. It takes less than 15 minutes and is painless.

b) Dietary history. This involves careful questioning by the dietitian to determine how much I eat at home. This may take 30-60 minutes.

c) Impedance plethysmography. I will lie down on a cot and have electrodes attached to my arms. The resistance of my body to a small electrical impulse indicates how much body fat I have. This procedure is painless and takes 5-10 minutes.

d) D20 ingestion. This is a special "heavy" water, which can be easily measured. I will drink some as a small glass of water. It has no harmful effects and tastes like normal water. For the next five (5) hours, my urine will be collected.

e) Screening blood tests. Two tablespoons of blood will be drawn.

f) Test dose of acetaminophen (Tylenol) 650 mg and sulfamethoxazole (Gantanol) 500 mg. These will be given to me orally shortly after I arrive. My urine will be collected for the next 5 hours.

The visit on this day will last 5-6 hours and will be scheduled at my convenience.

3. I will than be scheduled to enter the GCRC for three (3) separate 24 hour (overnight) admissions, each at least a week apart. The three admissions will involve identical protocols on my part.

4. I will enter the GCRC for each admission between 10:00 AM and Noon on the day scheduled. Lunch and supper will be served and I can eat without any restrictions. After 8:00 PM, I cannot eat any food or drink calorie-containing beverages until the next morning's meal. I will tell the dietitian my choices for the next day's meal at this time also. From this point on, the following procedures will be performed:

a) Either at 8:00 PM or 8:00 AM the next morning, an intravenous line will be placed. This will stay in place until 6:00 PM the next day.

b) I will have a sample of my breath collected, which involves blowing into a small rubber tube and syringe apparatus for a few seconds.

c) Beginning during the night or the next morning, the nurse will begin infusions of special "heavy" sugars or others nutrients into the intravenous line. These contain no radioactivity nor is there any radioactivity involved at any time in this study. Each admission will involve infusions of different sugars or other nutrients (glucose, galactose, palmitate, acetate). These are given in "tracer" quantities, which means that the amount given is so small that it will not itself affect my metabolism. The intravenous solution will also contain acetaminophen (Tylenol), which will be given at 100 mg/hr. This is roughly the same dose of acetaminophen (Tylenol) that I might take orally for a cold or a head-ache. The infusion will continue until 6:00 PM.

d) I will also take one teaspoon (500 mg) of the antibiotic sulfamethoxazole (Gantanol) starting at midnight for 4 total doses (midnight, 4 AM, 8 AM, 1 PM) or starting at 10 AM for 3 total doses (10 AM, 1 PM, 4 PM). This is the approximate dose I would take of this medication if I had a urinary tract infection, bronchitis or other mild infection (sulfamethoxazole is present in the medications Bactrim and Septra, which are commonly used for various infections).

e) I will collect all my urine after midnight in the containers provided.

f) Between 7:30 and 9:00 AM, a second IV line will be placed for blood drawing, to avoid my having to get needle sticks every hour. Each hour from 8:00 or 9:00 AM, a sample of blood is drawn (less than 2 teaspoons) and a breath sample is collected, in addition to saving all my urine, until 6:00 PM.

g) Each hour from 8:00 AM, I will breath into a mask or tube for a few minutes to measure my oxygen use and carbon dioxide production.

h) I will be fed in the morning one of three ways--either with a breakfast, with Ensure (a liquid diet formula) or with intravenous sugar water (glucose). Breakfast or brunch will be served at 10:30 AM in accordance with my requests from the previous evening. I agree to try to eat the entire meal that is served. I can drink beverages of my choice during this meal, but they can contain no caffeine. Ensure feedings will

begin either at 8:00 or 9:00 AM and continue hourly until 5:00 PM. There are several flavors which I may choose from (vanilla, strawberry, chocolate). Each hourly serving will be about 10 ounces. Intravenous sugar water (glucose) will begin at 8:00 AM and go until 6:00 PM. In my case, feeding will be with _____.

i) At 5:00 PM or 6:00 PM, the infusion will be discontinued and the first intravenous line removed. After the 6:00 PM samples are taken, the second intravenous line will be removed. The admission will be completed at this time (6:00 PM).

j) I may eat supper at this time in the GCRC and then leave or I may leave immediately at 6:00 PM.

C. Risks/Discomforts

1. Procedures. A hematoma (black and blue mark can result from placement of intravenous lines. This can be painful but carries no significant risks. Infections from intravenous lines rarely occur from such brief (24 hour) placement. However, any signs of inflammation (redness, tenderness, warmth of the skin over the catheter) will lead to removal of the catheter and treatment with warm soaks and elevation. The other procedures in these studies (breath and urine collections, diet) are not associated with any risks.

2. Acetaminophen administration. Acetaminophen (Tylenol) is given at 100 mg per hour for 15 hours or less. This is less than or similar to the usual oral dose taken in over-the counter pills. The experience of the investigators with large numbers of volunteers receiving intravenous acetaminophen has revealed no problems or intolerance. The one area of potential concern is for subjects taking azidothymidine (AZT). Tylenol should not be given to anyone taking AZT, because it can worsen potential side-effects of AZT on the liver. For this reason, if I am taking AZT, I will stop it during each GCRC admission, so that it is temporarily not present in my system. Brief periods without AZT are considered safe and should not decrease the efficacy of the AZT as a chronic prophylactic treatment in HIV infected individuals.

3. Sulfamethoxazole administration. If I have ever experienced an allergic reaction to a sulfa drug in the past, I will be excluded from this study. If I develop evidence of allergy during any study (rash, hives, shortness of breath) the drug will be halted immediately and treatment

for the allergy will be given. Many patients with AIDS given sulfa drugs (Bactrim, Septra) for pneumonia develop a rash after a week or so on treatment. However, this rash should not occur with brief treatment such as that given in this study (less than 24 hours of treatment). Also, the rash goes away when the medicine is stopped and has no permanent or long-term consequences. More serious allergic responses to sulfa drugs have not been reported in AIDS patients.

4. Stable isotope administration. The special "heavy" molecules I will receive contain no radioactivity and have no recognized adverse effects.

5. Blood drawing. The total amount of blood drawn over the 3 infusions will be 250 ml over the course of at least 3 weeks. This amount is well within the Red Cross' guidelines (less than 450 ml per month). If I have a low red blood cell count at any time during the study, I will be excluded from participating. I have been advised not to donate blood for at least 8 weeks following the completion of this study because of the blood drawing during this study.

6. Potential loss of privacy. Participation in research may involve loss of privacy. My research records will be handled as confidentially as is possible within the law. All records will be coded. No individual identities will be used in any reports or publications resulting from this study. While confidentiality cannot be guaranteed, it will be protected up to the full extent of the law.

D. Treatment and Compensation for injury

If I am injured as a result of being in this study, treatment will be available. The cost of such treatment may be covered by the University of California depending on a number of factors. The university does not normally provide any other form of compensation for injury. For further information, I may call the office of the Committee on Human Research at (415) 476-1814.

E. Benefits

I may benefit from participating in the study, by receiving nutritional education and information regarding my actual food intake. However, no benefit can be guaranteed to me.

F. Alternatives

The alternative is not to participate in the study.

G. Costs

There will be no charges to me from this study.

H. Reimbursement

I will receive $250 upon completion of the screening plus 3 GCRC admissions. I will receive a prorated portion of the $250 based on the percent of the study completed ($10 for the screening tests then $80 for each of the 3 admissions completed). If an extra admission is necessary, I will be paid $80 more.

I. Questions

This study has been explained to me by Dr. Hellerstein or _____ and my questions were answered. If I have any other questions about the study I may call Dr. Hellerstein at (415) 821-8982 or 642-0646.

J. Consent

I have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

Participation in research is voluntary. I have the right to decline to participate or to withdraw at any point in this study without jeopardy to my medical care.

If I wish to participate, I should sign below.

_____

Date

Subject's signature

_____

Person obtaining consent

APPENDIX C

Consent Form #3

Subjects with AIDS

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
SAN FRANCISCO GENERAL HOSPITAL
INVESTIGATIONAL PROTOCOL

Consent to be a Research Subject

Study Title: Metabolic pathways, monokines and wasting in HIV infection.

A. Purpose and Background

Dr. Marc Hellerstein and his associates from the Department of Medicine at the University of California at San Francisco are conducting a study to investigate the metabolic causes of weight loss and appetite loss in Human Immunodeficiency Virus (HIV) infection. It is well recognized that many patients with HIV infection, with or without Acquired Immunodeficiency Syndrome (AIDS) lose weight with changes in body content of muscle, fat, vitamins, minerals, etc. The resulting malnutrition may cause further suppression of the immune system. For this reason, it is important to understand the causes of malnutrition in HIV infection. At present, however, the causes are not well understood. This study will attempt to determine whether metabolism of carbohydrates and fats is disordered in HIV infection, what hormones cause these abnormalities, and whether this could explain the weight loss and appetite loss. I have been asked to participate in this study because I have been diagnosed as having AIDS and have lost weight.

B Procedures

If I agree to participate in this study, the following will happen:

1. I will be evaluated to see if I am eligible to be in this study. This includes: a history of my medical condition, a brief physical examination and some blood tests (two tablespoons of blood will be drawn).

2. If I am eligible to participate based on the above evaluation, I will come to the General Clinical Research Center (GCRC) of the San Francisco General Hospital one morning for pre-study testing. On this day, I will have my skinfold thickness determined by calipers, my diet history taken by a research dietitian, my body composition determined by impedance plethysmography and D20 ingestion, screening blood tests drawn, and a test dose of acetaminophen (Tylenol) and sulfamethoxazole (Gantanol) given by mouth. Each of these will now be described:

a) Caliper measurements. This involves determining the thickness of my skinfolds to see how much fat I have at different areas of my body. It takes less than 15 minutes and is painless.

b) Dietary history. This involves careful questioning by the dietitian to determine how much I eat at home. This may take 30-60 minutes.

c) Impedance plethysmography. I will lie down on a cot and have electrodes attached to my arms. The resistance of my body to a small electrical impulse indicates how much body fat I have. This procedure is painless and takes 5-10 minutes.

d) D20 ingestion. This is a special "heavy" water, which can be easily measured. I will drink some as a small glass of water. It has no harmful effects and tastes like normal water. For the next five (5) hours, my urine will be collected.

e) Screening blood tests. Two tablespoons of blood will be drawn.

f) Test dose of acetaminophen (Tylenol) 650 mg and sulfamethoxazole (Gantanol) 500 mg. These will be given to me orally shortly after I arrive. My urine will be collected for the next 5 hours.

The visit on this day will last 5-6 hours and will be scheduled at my convenience.

3. I will than be scheduled to enter the GCRC for three (3) separate 24 hour (overnight) admissions, each at least a week apart. The three admissions will involve identical protocols on my part.

4. I will enter the GCRC for each admission between 10:00 AM and Noon on the day scheduled. Lunch and supper will be served and I can eat without any restrictions. After 8:00 PM, I cannot eat any food or drink calorie-containing beverages until the next morning's meal. I will tell the dietitian my choices for the next day's meal at this time also. From this point on, the following procedures will be performed:

a) Either at 8:00 PM or 8:00 AM the next morning, an intravenous line will be placed. This will stay in place until 6:00 PM the next day.

b) I will have a sample of my breath collected, which involves blowing into a small rubber tube and syringe apparatus for a few seconds.

c) Beginning during the night or the next morning, the nurse will begin infusions of special "heavy" sugars or others nutrients into the intravenous line. These contain no radioactivity nor is there any radioactivity involved at any time in this study. Each admission will involve infusions of different sugars or other nutrients (glucose, galactose, palmitate, acetate). These are given in "tracer" quantities, which means that the amount given is so small that it will not itself affect my metabolism. The intravenous solution will also contain acetaminophen (Tylenol), which will be given at 100 mg/hr. This is roughly the same dose of acetaminophen (Tylenol) that I might take orally for a cold or a head-ache. The infusion will continue until 6:00 PM.

d) I will also take one teaspoon (500 mg) of the antibiotic sulfamethoxazole (Gantanol) starting at midnight for 4 total doses (midnight, 4 AM, 8 AM, 1 PM) or starting at 10 AM for 3 total doses (10 AM, 1 PM, 4 PM). This is the approximate dose I would take of this medication if I had a urinary tract infection, bronchitis or other mild infection (sulfamethoxazole is present in the medications Bactrim and Septra, which are commonly used for various infections).

e) I will collect all my urine after midnight in the containers provided.

f) Between 7:30 and 9:00 AM, a second IV line will be placed for blood drawing, to avoid my having to get needle sticks every hour. Each hour from 8:00 or 9:00 AM, a sample of blood is drawn (less than 2 teaspoons) and a breath sample is collected, in addition to saving all my urine, until 6:00 PM.

g) Each hour from 8:00 AM, I will breath into a mask or tube for a few minutes to measure my oxygen use and carbon dioxide production.

h) I will be fed in the morning one of three ways--either with a breakfast, with Ensure (a liquid diet formula) or with intravenous sugar water (glucose). Breakfast or brunch will be served at 10:30 AM in accordance with my requests from the previous evening. I agree to try to eat the entire meal that is served. I can drink beverages of my choice during this meal, but they can contain no caffeine. Ensure feedings will

begin either at 8:00 or 9:00 AM and continue hourly until 5:00 PM. There are several flavors which I may choose from (vanilla, strawberry, chocolate). Each hourly serving will be about 10 ounces. Intravenous sugar water (glucose) will begin at 8:00 AM and go until 6:00 PM. In my case, feeding will be with _____.

i) At 5:00 PM or 6:00 PM, the infusion will be discontinued and the first intravenous line removed. After the 6:00 PM samples are taken, the second intravenous line will be removed. The admission will be completed at this time (6:00 PM).

j) I may eat supper at this time in the GCRC and then leave or I may leave immediately at 6:00 PM.

C. Risks/Discomforts

1. Procedures. A hematoma (black and blue mark can result from placement of intravenous lines. This can be painful but carries no significant risks. Infections from intravenous lines rarely occur from such brief (24 hour) placement. However, any signs of inflammation (redness, tenderness, warmth of the skin over the catheter) will lead to removal of the catheter and treatment with warm soaks and elevation. The other procedures in these studies (breath and urine collections, diet) are not associated with any risks.

2. Acetaminophen administration. Acetaminophen (Tylenol) is given at 100 mg per hour for 15 hours or less. This is less than or similar to the usual oral dose taken in over-the counter pills. The experience of the investigators with large numbers of volunteers receiving intravenous acetaminophen has revealed no problems or intolerance. The one area of potential concern is for subjects taking azidothymidine (AZT). Tylenol should not be given to anyone taking AZT, because it can worsen potential side-effects of AZT on the liver. For this reason, if I am taking AZT, I will stop it during each GCRC admission, so that it is temporarily not present in my system. Brief periods without AZT are considered safe and should not decrease the efficacy of the AZT as a chronic prophylactic treatment in HIV infected individuals.

3. Sulfamethoxazole administration. If I have ever experienced an allergic reaction to a sulfa drug in the past, I will be excluded from this study. If I develop evidence of allergy during any study (rash, hives, shortness of breath) the drug will be halted immediately and treatment for the allergy will be given. Many patients with AIDS given sulfa drugs (Bactrim, Septra) for pneumonia develop a

rash after a week or so on treatment. However, this rash should not occur with brief treatment such as that given in this study (less than 24 hours of treatment). Also, the rash goes away when the medicine is stopped and has no permanent or long-term consequences. More serious allergic responses to sulfa drugs have not been reported in AIDS patients.

4. Stable isotope administration. The special "heavy" molecules I will receive contain no radioactivity and have no recognized adverse effects.

5. Blood drawing. The total amount of blood drawn over the 3 infusions will be 250 ml over the course of at least 3 weeks. This amount is well within the Red Cross' guidelines (less than 450 ml per month). If I have a low red blood cell count at any time during the study, I will be excluded from participating. I have been advised not to donate blood for at least 8 weeks following the completion of this study because of the blood drawing during this study.

6. Potential loss of privacy. Participation in research may involve loss of privacy. My research records will be handled as confidentially as is possible within the law. All records will be coded. No individual identities will be used in any reports or publications resulting from this study. While confidentiality cannot be guaranteed, it will be protected up to the full extent of the law.

D. Treatment and Compensation for injury

If I am injured as a result of being in this study, treatment will be available. The cost of such treatment may be covered by the University of California depending on a number of factors. The university does not normally provide any other form of compensation for injury. For further information, I may call the office of the Committee on Human Research at (415) 476-1814.

E. Benefits

I may benefit from participating in the study, by receiving nutritional education and information regarding my actual food intake. However, no benefit can be guaranteed to me.

F. Alternatives

The alternative is not to participate in the study.

G. Costs

There will be no charges to me from this study.

H. Reimbursement

I will receive $250 upon completion of the screening plus 3 GCRC admissions. I will receive a prorated portion of the $250 based on the percent of the study completed ($10 for the screening tests then $80 for each of the 3 admissions completed). If an extra admission is necessary, I will be paid $80 more.

I. Questions

This study has been explained to me by Dr. Hellerstein or _____ and my questions were answered. If I have any other questions about the study I may call Dr. Hellerstein at (415) 821-8982 or 642-0646.

J. Consent

I have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

Participation in research is voluntary. I have the right to decline to participate or to withdraw at any point in this study without jeopardy to my medical care.

If I wish to participate, I should sign below.

_____

Date

Subject's signature

_____

Person obtaining consent