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De novo lipogenesis in humans: metabolic and regulatory aspects

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The enzymatic pathway for converting dietary carbohydrate (CHO) into fat, or de novo lipogenesis (DNL), is present in humans, whereas the capacity to convert fats into CHO does not exist. Here, the quantitative importance of DNL in humans is reviewed, focusing on the response to increased intake of dietary CHO. Eucaloric replacement of dietary fat by CHO does not induce hepatic DNL to any substantial degree. Similarly, addition of CHO to a mixed diet does not increase hepatic DNL to quantitatively important levels, as long as CHO energy intake remains less than total energy expenditure (TEE). Instead, dietary CHO replaces fat in the whole-body fuel mixture, even in the post-absorptive state. Body fat is thereby accrued, but the pathway of DNL is not traversed; instead, a coordinated set of metabolic adaptations, including resistance of hepatic glucose production to suppression by insulin, occurs that allows CHO oxidation to increase and match CHO intake. Only when CHO energy intake exceeds TEE does DNL in liver or adipose tissue contribute significantly to the whole-body energy economy.

It is concluded that DNL is not the pathway of first resort for added dietary CHO, in humans. Under most dietary conditions, the two major macronutrient energy sources (CHO and fat) are therefore *not* interconvertible currencies; CHO and fat have independent, though interacting, economies and independent regulation. The metabolic mechanisms and physiologic implications of the functional block between CHO and fat in humans are discussed, but require further investigation.

Introduction

In this review, I will address the fate of surplus dietary carbohydrate (CHO) in humans. More specifically, the focus will be on conversion of CHO to fat, or de novo lipogenesis (DNL), with the question framed in quantitative terms: to what extent is surplus dietary CHO energy converted to fat? The various ways in which CHO content of the diet can be increased will be considered: increased CHO that replaces dietary fat (high-CHO low-fat, eucaloric diets); CHO added to a mixed diet, where CHO energy is less than total energy expenditure (TEE) but total energy intake exceeds TEE; and CHO consumption in excess of TEE. This review will therefore focus on the upper limits and consequences of increased CHO intake rather than on the lower limits and consequences of insufficient fat intake.

Background and historical review

The enzymatic pathway of DNL is present in all organisms. Knowledge concerning the genes and enzymes of DNL and their regulation has advanced considerably (reviewed in Bloch, 1977; Girard *et al*, 1994; Sul *et al*, 1993). Nevertheless, quantitative and regulatory aspects of DNL in metabolic physiology remain controversial. It has been widely presumed that DNL functions primarily as a sink for storage of excess CHO energy and to a lesser extent for the synthesis of structural, non-essential lipids (Table 1). Indeed, many animals are well known to convert CHO to fat (Lawes & Gilbert, 1886; Florkin & Stotz, 1977): pigs fatten on a grain diet, for example, and bees convert honey to wax. Although conversion of CHO to fat prior to oxidation is believed to be thermogenically costly (using ca. 28% of the energy content of CHO, Flatt, 1978; Hellerstein *et al*, 1996), the pathway is known to have regulated steps and is therefore presumed to play a role in normal physiology. Despite considerable information about the regulation of acetyl-CoA carboxylase, fatty acyl synthetase, malonyl-CoA and other components of the enzymatic pathway (Bloch, 1977; Girard *et al*, 1994), the quantitative importance of DNL has remained an area of uncertainty until recently.

Most of this uncertainty can be attributed to limitations in the methods available to address this question. Both indirect and direct techniques for measuring DNL have been used.

Indirect approaches for assessing the role of DNL in humans

A number of indirect approaches have been applied to this question.

Comparison of fatty acid (FA) composition in adipose tissue and diet

Over 30 y ago, Hirsch (1965) observed that adipose FA composition in human subjects closely resembled that of the Western diet. Individuals were also placed on con-

Table 1 Overview of de novo lipogenesis (DNL) as a pathway

(A) Presumed functions of DNL in the organism:
- Synthesis of structural lipids
- Storage of surplus CHO energy as fat

(B) High thermogenic costs

(C) Enzymology and regulation of DNL:
- Key regulatory node = Acetyl-CoA carboxylase (ACC)
- Malonyl-CoA as product of ACC and regulator of FA oxidation

(D) Quantitative importance of DNL uncertain

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trolled diets of different FA compositions, for 6–12 months, to allow turnover of adipose FA depots. The adipose FA composition changed to resemble that of the new diet. These investigators concluded that the body adds little endogenous FA to the dietary mix, that is, we are what we eat with regard to whole body FA stores. The obvious inference from these findings is that DNL is not a quantitatively important pathway in humans, at least under conditions of Western (high fat) diets.

Other explanations of these results can also be put forward, however. It is possible that selectivity exists for storage (esterification) relative to oxidation (lipolysis and entry into mitochondria) for FA in humans. If this were the case, de novo FA could be synthesized and oxidized without accumulating in tissues. Nor do static changes in FA composition provide information about fluxes through pathways; for example, DNL/fat oxidation cycling could occur at a very high or a very low rate and remain consistent with these observations. Nevertheless, a similar principle (dilution of the essential FA linoleate in circulating lipids) has been used recently as a method for estimating rates of DNL (Petrek *et al*, 1997; Hudgins *et al*, 1996; see below).

Enzyme activities in tissues

The enzyme activities of the DNL pathway in adipose tissue and liver have been used to estimate an upper bound on *in vivo* lipogenesis. Most studies (Shrago *et al*, 1971; Weiss *et al*, 1986; Sjöstrom, 1973; Askanazi *et al*, 1980) have reported quite low maximal lipogenic rates in human lipogenic tissues compared to rodents, for example (Table 2). These studies might underestimate capacity for inducing DNL, however, since most subjects were studied under typical high-fat eucaloric conditions. Sjöstrom reported only modest stimulation of DNL enzymes in humans after a high CHO/low fat diet (Sjöstrom, 1973). During parenteral overfeeding some evidence has been presented (Askanazi *et al*, 1980) for induction of DNL enzymes in human adipose tissue, but the quantitative importance has not been established.

Indirect calorimetry to measure net DNL in the whole body

The most widely used technique for assessing DNL *in vivo* has been indirect calorimetry. The principles of gas exchange to measure fuel selection have been reviewed elsewhere (Jéquier *et al*, 1987; Elia & Livesey, 1988). This approach can be used to estimate net conversion of CHO to fat, since DNL is the only metabolic process with an RQ > 1.0. Although different estimates of the true RQ for palmitate synthesis have been provided (Hellerstein *et al*, 1996; Jéquier *et al*, 1987), it is clear that DNL within a biologic system will generate RQ > 1.0 and that RQ > 1.0 represents DNL. The major interpretative problem with indirect calorimetry in this regard is that it measures *net* DNL, not unidirectional flux through the pathway. An NP RQ > 1.0 indicates only that synthesis is greater than oxidation of fat in the whole system during the period that is sampled. Lipogenesis from CHO in one tissue could be balanced by fat oxidation in another, for example, and the resultant RQ (1.0) would not be distinguishable from direct CHO oxidation (Tappy *et al*, 1995).

Nevertheless, indirect calorimetry has provided useful information about the response to large CHO loads. A number of studies (Acheson *et al*, 1982, 1984; Hellerstein *et al*, 1991) have confirmed that a single meal containing large amounts of CHO energy (up to 500 g CHO) in previously weight stable subjects does not cause NP RQ to rise above 1.0. This has been interpreted as evidence against a quantitatively important role for DNL in the day-to-day storage of surplus CHO energy; also, storage as glycogen was concluded to represent the fate of excess dietary CHO. Several days of surplus energy intake as CHO (Schwarz *et al*, 1995; Passmore and Swindells, 1963) also does not induce much net DNL (RQ reaches 0.98–1.01). In contrast, studies of massive CHO overfeeding (6000 kcal/d; 1500 g CHO) performed by Acheson *et al* (1988) showed that after about three days, when a positive whole CHO balance of 800 g had occurred, RQ rose well above 1.0; these investigators calculated that maximal whole-body glycogen storage capacity was ca. 700–1000 g and needed to be exceeded before net DNL became significant, after which time substantial net DNL occurred (for example, 150 g/d net fat synthesis after seven days of overfeeding). These dietary studies were highly unphysiologic, however, and do not clarify the physiologic role of DNL under less extreme dietary stresses.

Stoichiometry (macronutrient intake vs changes in body composition)

The least ambiguous indirect evidence for DNL would come from stoichiometry: if the rate of accumulation of adipose fat is greater than the intake of dietary fat, there must have been at least that much DNL. The only study in humans using this approach was the fascinating report of the 'Guru Walla' overfeeding (Pasquet *et al*, 1992), a traditional fattening ritual in Cameroon wherein adolescent boys are deliberately overfed to an extraordinary degree. These young men ingest a high CHO diet (70%) containing 7000 kcal/d; they gain 12 kg fat over 10 weeks while eating

Table 2 Tissue enzyme activities in humans and rat[a]

	Liver		*Adipose*						
	Human	*Rat*	*Human*			*Rat*			
Enzyme			*pr*	*sc*	*om*	*pr*	*sc*	*om*	*ep*
PDH	1.5	16.4	—	2.0	—	—	—	—	23.6
ATP citrate lyase	1.0	0.8	0.9	n.d.	1.5	73.1	33.5	36.8	—
FA synthase	0.8	6.4	1.0	0.9	1.0	52.2	—	—	54.3

[a]Measurements shown in milliunits per milligram of protein. Abbreviations: pr, perirenal; sc, subcutaneous; om, omental; ep, epididymal; n.d., not detectable. Adapted from Shrago *et al*, 1971; Weiss *et al*, 1986. Data on human tissues were from subjects on typical Western diets (fat content up to 40%). No differences were observed after a high-carbohydrate diet for three days in these studies.

Table 3 'Guru Walla' Model—Summary

(1) Implications: Substantial DNL can be induced in humans, under extreme dietary conditions (CHO intake >> TEE)
(2) Unanswered questions:
- Threshold for inducing significant DNL?
- Tissue site of DNL?
- Diurnal cyclicity of DNL?
- Is absolute DNL > net fat accumulation?

a total of only 4 kg fat. Clearly, under these exceptional nutritional conditions where CHO intake (ca. 5000 kcal/d) greatly exceeds TEE, surplus CHO must be converted to fat, even though DNL was not directly measured. It must be concluded then that DNL in the whole body at a quantitatively significant rate is possible in humans. Many questions were not answered, however (Table 3): in what tissue(s) did the DNL occur? Was there even greater unidirectional DNL than apparent by net accumulation of fat (was there a circadian rhythm of fat synthesis and subsequent oxidation)? What was the threshold for total CHO surplus after which DNL was induced? Are there genetic differences for DNL between this ethnic group and other human populations? And, do people ever *voluntarily* ingest enough surplus CHO to induce significant DNL; if not, why not? The answer to this last question may provide important insights into the overall regulation of energy balance in humans, including the integration of food intake with nutrient stores.

Direct measurement of DNL in vivo in humans using isotopes

The direct approach for measuring flux through the DNL pathway or any metabolic pathway *in vivo* generally requires the use of isotopes. It is therefore necessary to discuss briefly a few technical points concerning isotopic measurement of DNL, before we can interpret physiologic results using this approach. Measurements of biosynthetic processes in general, not only DNL, but also protein synthesis, gluconeogenesis, polysaccharide synthesis, polynucleotide synthesis, etc., have presented substantial technical problems (reviewed in Hellerstein & Neese, 1992; Waterlow *et al*, 1978; Katz, 1985; Dietschy & Brown, 1974). The central and most intractable of these problems has been how to gain access to the degree of isotope labeling of the true biosynthetic precursor pool, in order to interpret isotope incorporation into the biosynthetic product as a chemical flux rate. For the case of lipogenesis, Dietschy & Brown (1974) pointed out over 20 y ago that errors in the measurement of cholesterol and FA synthesis rates are unavoidable when using labeled acetate incorporation, because of variable, unpredictable and often extensive intracellular dilution in the cytosolic acetyl-CoA pool.

Fortunately, over the past half-dozen years or so, two methods have been developed that resolve this problem and therefore allow reliable measurement of DNL. The two methods are mass isotopomer distribution analysis (MIDA) and ^2H_2O incorporation.

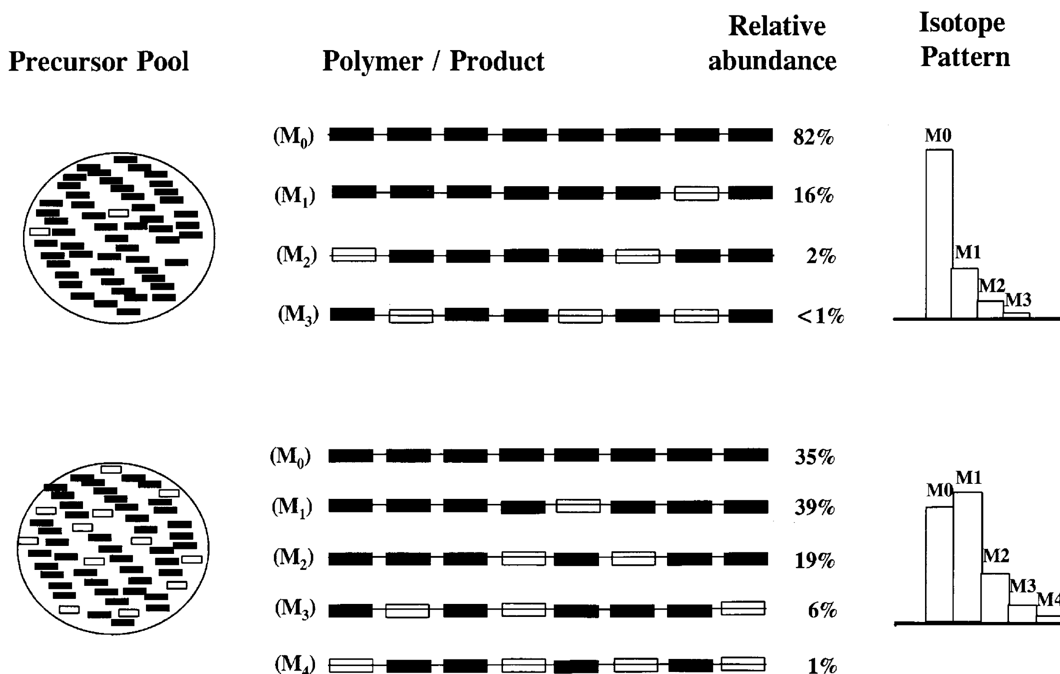
The MIDA technique has been described in detail elsewhere (Hellerstein *et al*, 1991, 1996; Hellerstein & Neese, 1992; Neese *et al*, 1995; Faix *et al*, 1993; Hellerstein, 1995). In brief, this is a stable isotope mass spectrometric method for measuring polymerization biosynthesis based on the principles of combinatorial probabilities. The basic concept is that polymers are composed of two or more repeating monomeric subunits, which are assembled from an intracellular biosynthetic precursor pool; and the quantitative pattern or distribution of labeled subunits in the polymer population reflects the isotopic enrichment of the monomeric pool, according to the binomial or multinomial expansion. It follows that analysis of the labeling pattern (the mass isotopomer distribution) in the polymer by quantitative mass spectrometry will reveal the intracellular precursor pool enrichment. Fractional biosynthesis can then be calculated by application of the precursor-product relationship (Hellerstein & Neese, 1992). A numerical example of combinatorial probabilities (Figure 1A) and a general scheme of mass isotopomer distributions (Figure 1B) are shown.

The ^2H_2O (labeled water) technique (Table 4) attempts to overcome the problem of metabolite compartmentalization and differential dilution by using H_2O, which is freely permeable across almost all biological membranes (other than distal renal tubule) and is well mixed in the body (Leitch & Jones, 1993; Jones *et al*, 1995). Labeled hydrogen from H_2O enters into biosynthetic pathways either by exchange with solvent H_2O or by specific incorporation of H from NAD(P)H (Jungas, 1968). If the number of hydrogen atoms incorporated from H_2O per mole of product is known, the isotopic enrichment of ^2H_2O or ^3H_2O from any body fluid can be used to calculate fractional biosynthesis by application of the precursor-product relationship. The major complication here is determining the actual H:C ratio present in a biosynthetic product in a particular tissue in a given experiment (Selmer & Grunnet, 1976; Lee *et al*, 1994). The H:C ratio has been determined accurately by use of MIDA (Lee *et al*, 1994), but this approach has not been applied in humans. The existence of an isotope effect, for ^3H_2O particularly but for ^2H_2O as well, represents another potential experimental artifact. Nevertheless, using literature values for H:C ratios, DNL has been measured in humans by this method (Leitch & Jones, 1993; Jones *et al*, 1995).

It is important to mention what might seem an obvious point: an investigator can only measure DNL in pools that can be experimentally sampled. If DNL occurs in a 'hidden' location, that is, inaccessible to collection by the investigator, it will not be measured. This caveat becomes important for interpretation if there is a possibility of newly synthesized TG being stored in the hepatic cytosolic pool rather than secreted as VLDL-TG, for example, or when adipose DNL might be occurring, since the enormous adipose TG pool size might dilute out recent incorporation of label and preclude measurement of DNL over the short-term (see below).

Regulation of DNL in humans

Results from both MIDA (Hellerstein *et al*, 1996; Hudgins *et al*, 1996; Hellerstein *et al*, 1991; Neese *et al*, 1995; Faix *et al*, 1993; Hellerstein, 1995) and ^2H_2O incorporation (Leitch & Jones, 1993; Jones *et al*, 1995) agree that hepatic DNL is a quantitatively minor pathway in men under conditions of a normal Western (high fat) diet. Calculated synthesis rate of palmitate or total non-essential FA based on flux through the circulating VLDL-TG pool is $<1-2$ g/d, a small amount compared to the $50-150$ g fat in the diet each day. These results confirm prior conclusions from indirect calorimetry (Tappy *et al*, 1995; Acheson *et al*,



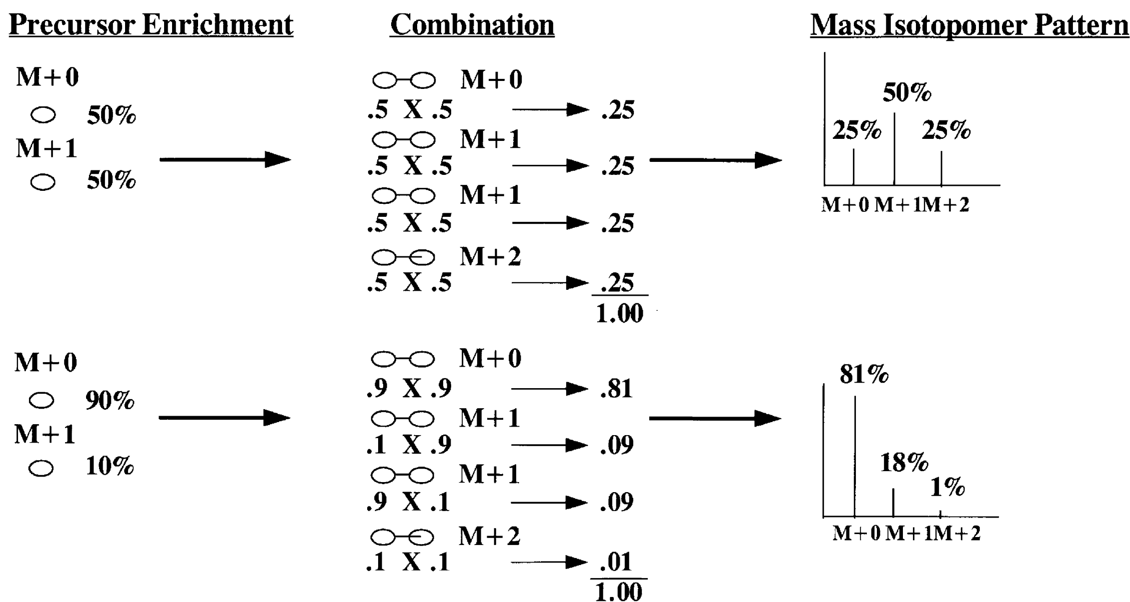


Figure 1A Schematic model of polymerization biosynthesis. The principles of combinatorial probabilities determine the pattern of ^{12}C and ^{13}C-containing subunits in newly synthesized polymers. In this simulation, natural abundance or 10% isotopically labelled precursor pools of a subunit ([1-^{13}C]-acetate) combine into a polymer of 8 subunits (palmitate). The population of each of these pools will contain a characteristic distribution of M0, M1, M2, etc. molecular species (mass isotopomers). These proportions can be represented as a frequency histogram of the mass isotopomer pattern in the polymer and can be measured by mass spectrometry. After correction for natural abundance, the degree of enrichment of the precursor pool can be calculated by comparing measured patterns of mass isotopomer abundances to those predicted from theoretical precursor pool enrichments.
Figure 1B Simple numerical example of MIDA principle. The combinatorial probabilities of unlabeled, single-labeled and double-labeled molecular species are shown for a hypothetical polymer composed of two subunits, with precursor pool labeling either 50% or 10%.

Table 4 ^2H_2O method for measuring biosynthesis of lipids

(1) Strategy: *Avoid* the problem of compartmentalization
(2) Complication: How does ^2H_2O get incorporated during biosynthesis? (Exchange with solvent H_2O? NAD(P)H?)
(3) Applications: DNL and cholesterogenesis
(4) Unresolved problem: true H:C ratio to use

1982, 1984; Hellerstein *et al*, 1991), comparisons of tissue to diet FA composition, and *in vitro* enzyme measurements (Shrago *et al*, 1971; Weiss *et al*, 1986; Sjostrom, 1973) (discussed above).

The initial studies were performed in healthy men who were non-obese, non-diabetic, and on high-fat, eucaloric diets in the absence of potential lipogenic stimulators such

as fructose or ethanol. All of the factors just noted have since been tested in humans, that is, the effects of gender, obesity, diabetes, medical illness, ethanol, fructose, low-fat diet and overfeeding. These results will now be reviewed.

DNL in women
Women have a different body composition than men, with larger percent body fat. Faix *et al* (1993) asked whether this might be due to a higher rate of DNL under *ad libitum* diet conditions. Young women were studied in the luteal and follicular menstrual phases. In the luteal phase, DNL was identical in these women as in healthy men (< 1–2 g/d). In the follicular phase, DNL was roughly three times elevated, and correlated to some extent with estradiol levels. The absolute rate of hepatic DNL could account for a rather small body fat burden (< 750 g fat per year), however, in comparison to dietary fat intake (> 35 kg fat per year). Although the signal (endocrine or dietary) responsible for higher DNL in the follicular menstrual phase is an interesting question, differences in DNL are unlikely to explain body composition differences between the genders.

DNL in obesity and diabetes
Obese, insulin resistant men exhibit modestly increased fractional DNL (Faix *et al*, 1993; Schwarz *et al*, 1993) while obese, normoinsulinemic men have normal DNL. The absolute rate of DNL could only account for about four extra grams of fat/day, however, which is not enough to account for body fat accrual. More likely, hyperinsulinemia stimulates hepatic DNL modestly. Consistent with this explanation, administration of insulin to poorly controlled diabetics causes an increase in DNL (Christiansen M, Neese R, Hellerstein M, unpublished observations).

DNL in inflammatory diseases
An interesting situation in which DNL is at least to some extent increased is during infection or inflammation, or in response to recombinant cytokines (such as interleukin-1 or -6, tumor necrosis factor, etc.) (Grunfeld & Feingold, 1992; Blackham *et al*, 1992; Hellerstein *et al*, 1993). These settings are also characterized by hypertriglyceridemia and reduced clearance of TG from plasma (Grunfeld & Feingold, 1992; Blackham *et al*, 1992; Hellerstein *et al*, 1993). Cytokine stimulation of DNL occurs only when there is liver glycogen present; prior fasting abolishes the stimulatory effect of tumor necrosis factor on hepatic DNL in rats (Blackham *et al*, 1992) and fed-state DNL is more elevated in AIDS patients than fasted-state DNL (Hellerstein *et al*, 1993). The stimulation of DNL by cytokines does not involve covalent modification of hepatic pyruvate dehydrogenase (PDH) or inhibition of PDH kinase activity (Blackham *et al*, 1992), therefore presumably reflects substrate-mediated activation of flux through PDH. Some workers have speculated that DNL is elevated as part of an adaptive hyperlipidemic response, which serves as a primitive host defense mechanism against infection (Grunfeld & Feingold, 1992). Nevertheless, from a quantitative point-of-view, total DNL again only represents a few grams of fat synthesized per day in illnesses such as AIDS (Hellerstein *et al*, 1993); body composition changes present in these conditions cannot be attributed to DNL.

DNL after ethanol (EtOH) ingestion
Siler *et al* (1996) measured hepatic DNL after administration of EtOH to normal men (24–48 g EtOH, equivalent to 2–4 alcoholic drinks). The fraction of circulating VLDL-palmitate contributed by DNL increased considerably, whether the EtOH was taken alone after an overnight fast or with a small meal (from < 4% to ca. 30%). Absolute DNL was still < 2 g after the 48 g EtOH dose, however, or < 5% of the administered EtOH dose. The great majority of EtOH was released from the liver as free acetate; 80% of EtOH clearance appeared in plasma as acetate. Important secondary consequences of increased plasma acetate flux were inhibition of FFA release into the circulation (Crouse *et al*, 1968) and reduction of whole body fat oxidation. A model of the fate and consequences of EtOH ingestion in normal humans is shown schematically (Figure 2) It is of interest to consider the metabolic consequences that resulted from DNL being only a minor quantitative sink for the 2-carbon load on the liver. Because DNL does not provide a significant disposal route, the liver releases free acetate, which has priority for oxidation by peripheral tissues, displaces FFA as a fuel and inhibits further release of FFA from adipose tissue. Thus, the liver converts EtOH to a substrate targeted to peripheral tissues' oxidative needs, rather than directly storing the 2-carbon load as fat. A similar model can be proposed for surplus CHO in the liver (see below).

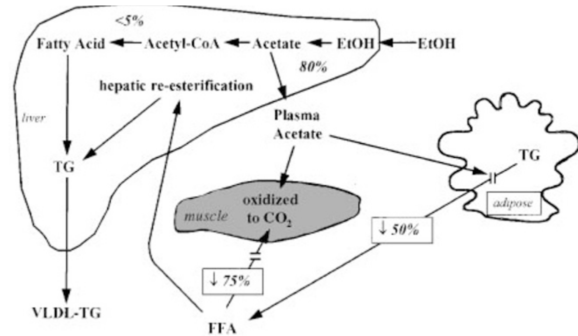


Figure 2 Key pathways of lipid metabolism in response to acute consumption of EtOH. Numbers shown reflect quantitative response to EtOH in normal human subjects (Siler *et al*, 1996).

DNL after intake of other potentially lipogenic substrates (fructose, medium chain TG)
Assimilation of fructose is almost exclusively by the liver and involves cleavage to triose-phosphates. In addition, high fructose diets can cause hypertriglyceridemia. For these reasons, fructose has been considered a potent stimulator of hepatic lipogenesis. Park *et al* (1992) quantified hepatic DNL in rats fed 70% fructose diets, using MIDA. Although DNL was somewhat increased and more hepatic PDH was in the active form due to inhibition of hepatic PDH kinase, only 15% of VLDL-palmitate came from DNL. The hypertriglyceridemia observed could not therefore be attributed to increased synthesis of FA from fructose. Schwarz *et al* (1993) tested the acute effects of a fructose load on hepatic DNL in human subjects, using MIDA. Compared to an equicaloric glucose load (each given at 10 mg/kg lean body mass/min, by the oral route for 6 h), fructose caused a qualitative induction of DNL, that is, the fraction of circulating palmitate derived from DNL rose to ca. 30%, but < 5% of the fructose load was converted to VLDL-FA. Although essentially 100% of a fructose load passes through the hepatic triose-phosphate pool (Neese *et al*, 1995), other fates of dietary fructose (for example, conversion to glucose or glycogen, release as

lactate) must have priority over conversion to fat. The lipogenic effects of medium-chain triglycerides are suggestive (Hill *et al*, 1990), but have not been studied quantitatively in animal models or in humans.

DNL on low-fat, high-carbohydrate eucaloric diets
It is important to distinguish between high-CHO (low-fat) eucaloric diets and surplus CHO hypercaloric diets. The former refers to the proportion of dietary energy represented by CHO, under conditions of neutral energy balance; the latter refers to the absolute quantity of CHO energy relative to total energy needs.

Parks *et al* (1998) and Hudgins *et al* (1996) have recently studied the effects of eucaloric, low fat:high CHO diets (15% fat: 70% CHO) on hepatic DNL in normal human subjects. Some interesting results have emerged. Either liquid or solid food high CHO diets that were composed predominantly of simple sugars (60% of CHO) induce marked fasting hypertriglyceridemia and a substantial increase in fractional DNL in the post-absorptive state. Solid food diets composed predominantly of complex CHO (60% of the CHO) also increase fasting serum triglycerides but do not increase fractional DNL (Hudgins *et al*, 1998). VLDL-triglycerides were documented to come almost 100% from plasma FFA on the complex CHO low-fat diets. Therefore, there may be 'lipogenic' and 'non-lipogenic' high CHO diets. Even the former diets, however, did not result in a high absolute rate of DNL (less than 10 g/day, by our calculations, Hudgins *et al*, 1996). Taken together, these results suggest that hepatic DNL may be involved in a regulatory or signaling manner in CHO-induced hypertriglyceridemia but that the absolute conversion rate of CHO to fat remains rather low on these eucaloric diets.

DNL during surplus CHO diets
Direct measurements therefore support the conclusion that hepatic DNL is generally not a quantitatively important pathway under normal dietary conditions in industrialized societies. But the question with which we began this review—to what extent is surplus CHO energy converted to fat through the DNL pathway?—is not addressed by the studies discussed so far. The true test of DNL (Table 1) requires a surplus of CHO energy intake; that is, CHO taken in excess of usual CHO oxidative needs in context of a total dietary energy intake greater than total energy expenditure (TEE).

It should be emphasized that, *a priori*, conversion to fat for storage as TG is *not* the only possible fate of surplus CHO energy, as just defined. 'Stoichiometric' arguments that extra CHO must be converted to fat are not valid (Table 5), unless CHO energy intake is by itself greater than TEE (minus protein intake) and occurs for long enough duration to fill whole body glycogen stores to their maximal capacity. Under other less extreme dietary conditions that nevertheless represent CHO overfeeding, the surplus CHO can be stored as glycogen or can replace fat in the whole-body fuel mixture (Table 5).

Table 5 Possible fates of surplus dietary CHO

(1) Storage as glycogen (liver, muscle)
(2) Conversion to fat (DNL in liver, adipose)
(3) Oxidation (replacement of other fuels, i.e. fat)

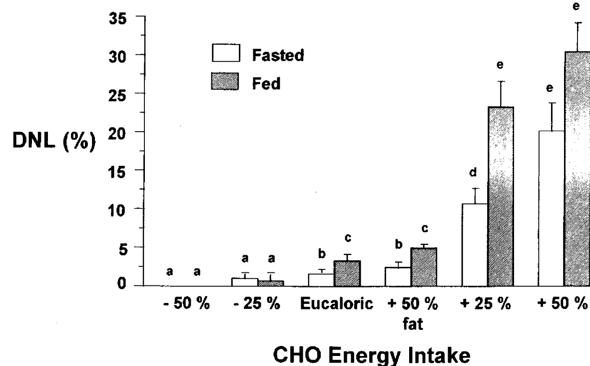


Figure 3 Hepatic DNL in fasted and fed states, in response to short-term alterations in dietary energy content. Fractional contribution from DNL to palmitate isolated from circulating VLDL-fatty acids was determined by MIDA (Hellerstein *et al*, 1991; Hellerstein & Neese, 1992). Values not sharing a common superscript are significantly different (from Schwarz *et al*, 1995).

A number of overfeeding studies have been performed in human subjects, providing surplus energy of various degrees, durations and compositions, while measuring hepatic DNL. It is useful to divide studies into those in which CHO energy was not greater than TEE and those in which it was.

Total CHO energy not in excess of TEE. In the first category, Neese *et al* (1994) and Schwarz *et al* (1995) measured hepatic DNL by MIDA during *ad libitum* oral overfeeding and controlled oral overfeeding, respectively, in subjects confined to a metabolic ward. Subjects in the former study self-selected an *ad libitum*, energy surplus diet for 7–14 d and took in close to 700 g CHO and 4500 kcal/d. The fractional contribution from DNL to VLDL-FA rose considerably (to 20–30% for palmitate) but represented only an estimated 1 g fat synthesized per day. Whole body NP RQ generally was not greater than 1.0 (or only slightly so) but stayed in the 0.95–1.02 range. In a follow-up study, Schwarz *et al* (1995) controlled dietary intake, comparing surplus or deficient CHO intake for 5 d periods (at +25%, +50%, −25% and −50% of baseline energy intake) to eucaloric and to surplus fat (50%) diets. Fractional DNL (the fraction of VLDL-FA derived from DNL) and absolute DNL rates were measured. A close relationship between recent dietary CHO energy and fractional DNL was observed (Figure 3). Indeed, measurement of fractional DNL was able to correctly identify almost everyone's recent dietary CHO intake. Stimulation of fractional DNL was specific for dietary CHO surplus: the +50% fat diets showed no effect on DNL (Figure 3). Nevertheless, absolute hepatic DNL even on the +50% CHO diet remained quantitatively insignificant (Table 6), representing ca. 3.3 g fat synthesized per day or 9.3 g/d CHO converted into fat, out of an added dietary CHO intake in the range of 300–400 g/d.

What was the fate of the added CHO energy, then, if not conversion to fat? The answer is apparent from Table 7: whole-body fuel selection adapted to dietary CHO energy. Therefore, even after an overnight fast, NP RQ was 0.95 ± 0.01 on +50% CHO diets, indicating replacement of most fat in the fuel mixture by CHO, despite being in the fasted state. Only 0.5 mg fat were oxidized per hour after an overnight fast (reduced from 3.6 g fat/h under eucaloric

Table 6 Absolute DNL (+50% CHO diet, $n = 6$)

Fractional DNL (%)	*Ks* (Hr^{-1})	$T\frac{1}{2}$ (*Hr*)	*[TG]* (*mg/dL*)	*TG Prod.* (*g/d*)	*Abs. DNL* (*g/d*)	*Glc to fat* (*g/d*)
25.4 ± 3.1	0.370 ± 0.059	2.21 ± 0.44	104 ± 7	28.0 ± 5.0	3.3 ± 0.8	9.3 ± 2.3

Fractional DNL, fraction of palmitate in VLDL derived from DNL; Ks, rate constant of VLDL-TG turnover; abs. DNL, absolute rate of DNL; Glc to fat, absolute rate of glucose conversion to fat (from Schwarz *et al*, 1995).

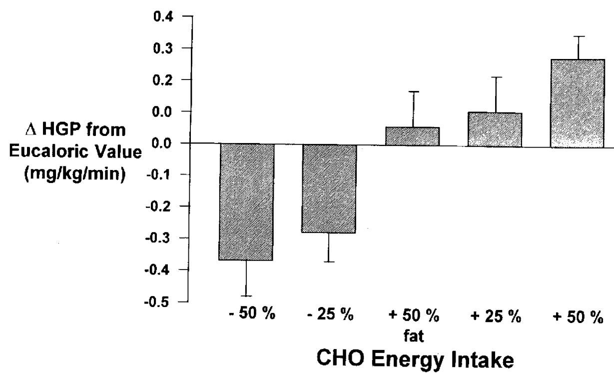


Figure 4 Fasting hepatic glucose production (Ra glucose) in response to short-term alterations in dietary energy content. Values shown are percent change from baseline (eucaloric) value, in mg/kg/min (from Schwarz *et al*, 1995).

Table 7 Whole body fuel utilization on different diets (NPRQ)

Eucaloric	*+50% CHO*	*−50% CHO*	*+25% CHO*	*−25% CHO*	*+50% fat*
0.84 ± 0.01	0.95 ± 0.01	0.77 ± 0.01	0.91 ± 0.01	0.80 ± 0.01	0.84 ± 0.02

conditions and 5.4 g fat/h during −50% CHO diet). Associated with the change in fuel selection was a suppression of adipose lipolysis, significantly increased serum insulin concentrations, reduced percent of lipolytic flux oxidized, and perhaps most importantly, increased hepatic glucose production (HGP, Figure 4). The difference in HGP between five days of +50% CHO and −50% CHO energy intake was more than 40% (from 1.76 ± 0.09 to 2.48 ± 0.13 mg/kg/min), or roughly the difference between normal and diabetic subjects (Hellerstein, 1995). Hyperglycemia did not result, however, in this setting of CHO overfeeding. Instead a high-flux (glucose-producing/glucose-oxidizing), normoglycemic fuel economy was observed.

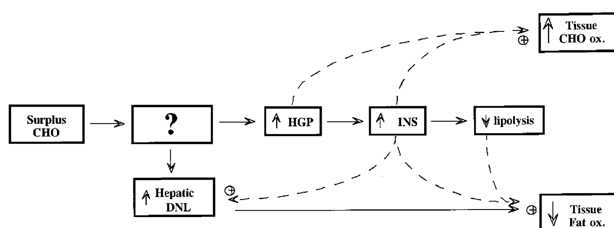


Figure 5 Hypothetical model of metabolic mechanisms responsible for sensitivity of whole-body fuel selection to dietary CHO intake. All precesses other than the box labeled '?' were measured (Schwarz *et al*, 1995). INS, insulin; ox, oxidation.

A schematic model of the metabolic response to surplus CHO energy not in excess of TEE can be proposed from these results (Figure 5). The key feature of this hypothesized response is that a signal of CHO stores (perhaps liver glycogen) results in increased HGP, which secondarily increases fasting insulin secretion; the combination of elevated glucose plus insulin alters fuel availability and fuel selection by peripheral tissues. Stimulation of DNL is observed, but does not play a major quantitative role in the altered macronutrient fluxes, other than perhaps a regulatory role in reducing tissue FA oxidation (McGarry & Foster, 1980).

Total CHO energy in excess of TEE. An important recent study in the second category was reported by Aarsland *et al* (1996). They administered iv plus nasogastric glucose at rates markedly above TEE for 10 d. Hepatic DNL (fractional and absolute) was measured by MIDA during a 10 h infusion of [^{13}C]-acetate. After 4−7 d of glucose overfeeding, hepatic DNL was stimulated 10-fold from baseline but remained $< 1/30$ of the whole body value calculated by indirect calorimetry.

What might explain this surprising result, wherein *net* whole-body DNL was greater than unidirectional hepatic DNL? Errors in the precursor pool estimate by MIDA are not a plausible explanation: hepatic acetyl-CoA enrichments would have to be systematically overestimated by 30-fold (that is, true precursor enrichments of 0.3% instead of the 9.0% calculated) and from a technical point-of-view, the mass spectrometric analyses are more reliable at the high enrichments in these studies than under conditions of lower DNL. The most reasonable interpretation of these results is that iv glucose overfeeding induces DNL in a 'hidden compartment'. Aarsland *et al* (1996) concluded that adipose DNL must be occurring. This may be true, or it is also possible that the 10 h isotope infusion was of insufficient duration to allow export of newly synthesized TG from the hepatic cytosolic TG pool, if the hepatic TG pool is expanded under these conditions. Neither possibility has been evaluated experimentally.

There have been no studies reported, to date, of even longer periods of CHO overfeeding at rates in excess of TEE (that is, comparable to the Guru Walla model, Pasquet *et al*, 1992) with direct measurement of liver or adipose tissue DNL.

Are surplus CHO calories not in excess of TEE therefore 'free' of risk for adding to body fat stores? It is important to be clear about the effects of surplus CHO energy not in excess of TEE on whole body fat balances, if DNL is not a quantitatively important pathway. This has been widely misinterpreted in the lay literature. Although surplus CHO energy at these levels may not be converted directly to fat

Table 8 Conclusions: what happens when surplus CHO is consumed?

(1) CHO not converted to fat (mysterious block)
(2) Instead: CHO burnt, fat not; CHO stores fill up
(3) Provides a system for matching fuel selection to recent CHO intake (and perhaps controlling intake)
(4) System wouldn't work if a safety-valve (CHO to fat) were open

in large quantities, CHO replaces fat as fuel by the body; total fat oxidation can be almost completely turned off by intake of surplus CHO (Table 7). Body fat is thereby spared by surplus CHO; despite the absence of DNL, body fat can accumulate, as long as there is any fat in the diet.

Metabolic response to surplus CHO energy intake in humans: the emerging model

Although a number of questions remain unresolved concerning the threshold for and tissue site of DNL in humans in response to long-term surplus CHO energy, the results to date allow a model to be proposed (Table 8, Figure 5).

Several points deserve emphasis:

1. DNL is not the 'pathway of first resort' in response to intake of surplus CHO. The first and quantitatively most important responses to increased CHO intake are increased glycogen storage and increased whole body CHO oxidation, not increased CHO conversion to fat. Although net fat balance and cumulative indirect calorimetry can not distinguish between direct CHO oxidation and lipogenesis/fat oxidation over the course of 24 h, and RQ must equal FQ under conditions of energy balance whether or not DNL occurs (as discussed by Flatt, 1987), there are extremely important functional consequences of the particular organization that appears to have evolved. Alternative systems could exist: there *could* have been a circadian cycle for lipogenesis and fat oxidation, for example (Figure 6), with storage of CHO as fat during the daytime then mobilization and oxidation of fat at night. The net result might be the same under conditions of energy balance, but the specific pathways involved and the possible disorders of their regulation (diseases) would be quite different. Instead, CHO energy taken in excess of postprandial energy requirements is stored in modestly expandable tissue glycogen stores during the daytime and is then released from glucose producing tissues (the liver) at a rate proportional to its prior accumulation, for oxidation during the overnight fast. Rather than observing highly cyclic values of RQ and a diurnal sequence of fat storage followed by fat oxidation in the presence of CHO surpluses, cyclic variations in RQ are damped and the system settles into a stable CHO oxidizing mode (Figure 6). The diurnal cycle consists of daytime CHO storage and night-time CHO release, rather than daytime DNL followed by night-time fat oxidation.

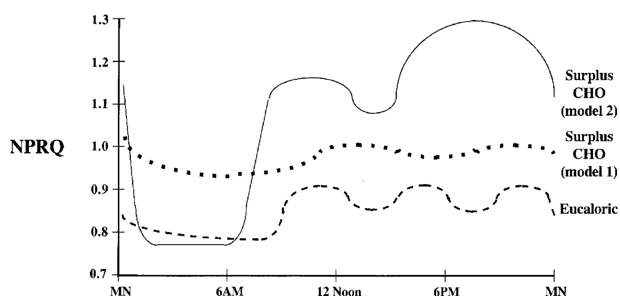


Figure 6 Circadian pattern of fuel selection (expressed as NPRQ): potential models of response to surplus dietary CHO. In model 1, NPRQ is close to 1.0 and relatively constant over the circadian feeding cycle. In model 2, NPRQ varies considerably in response to feeding or fasting, with low NPRQ during the overnight post-absorptive phase and NPRQ > 1.0 during the daytime absorptive phase.

Viewed from this perspective, the macronutrient regulatory system appears to be organized to match fuel selection to recent CHO intake. It has been long recognized (Cahill, 1976) that animals must reduce CHO oxidation in times of energy starvation, to preserve lean body mass (by reducing the need for gluconeogeneses from amino acids). The converse adaptation seems also to apply, namely, that CHO oxidation is increased in times of CHO surplus. Moreover, both adaptations involve the liver and modulation of HGP, rather than just direct oxidation of expanded or reduced CHO stores in peripheral tissues.

2. Many functional consequences can be predicted from point #1. A number of functional consequences for human metabolic physiology and disease can be predicted *a priori* from the simple notion that CHO is not readily converted to fat and that CHO stores in the body are limited, as Flatt has discussed elsewhere (Flatt, 1987). Many of these predictions have experimental support, as well:

(i) Whole body fuel selection is controlled by and responsive to recent CHO intake (and CHO stores in the body). This applies not only under conditions of macronutrient and energy balance, but also during periods of surplus (or deficient) CHO intake, that is, if CHO stores are changing.
(ii) HGP must be modulated in response to the balance between dietary CHO and whole body CHO oxidation. Because 24 h CHO oxidation must reflect total CHO intake but food intake is cyclic, glucose release at night (the interprandial period) must be sensitive to CHO intake during the day (the prandial period) (Figure 4). As a result, hepatic insulin sensitivity or pancreatic insulin secretion must be capable of modulation and must respond to diet. Without variable HGP, matching of 24 h fuel selection to dietary CHO would not work. Therefore, HGP is not a constant, like serum sodium concentration or osmolality, but depends upon recent CHO balances. Studies in rats (Neese *et al*, 1995), normal humans (Schwarz *et al*, 1995; Clore *et al*, 1995) and type II diabetes mellitus (Wing *et al*, 1994; Kelley *et al*, 1993; Christiansen *et al*, 1995) have documented the responsiveness of HGP to recent CHO energy intake. Biochemical mechanisms for this adaptation have been proposed. Liver glycogen content affects its own turnover by increasing sensitivity to glycogen phosphorylase (Hers, 1976). Therefore, expanded hepatic glycogen content increases HGP and contracted glycogen content reduces HGP (Schwarz *et al*, 1995; Clore *et al*, 1995; Neese *et al*, 1995). Insulin secretion has also been reported to be influenced by body CHO stores (Lilavivathana *et al*, 1978). Stated differently, the need for modulable HGP in response to high dietary CHO represents a form of physiologic (adaptive,

programmed) hepatic insulin resistance. The implication for diseases related to insulin resistance, including type II diabetes, have not been fully explored, although this metabolic adaptation appears to represent the physiologic basis of the therapeutic response to energy restriction ('weight loss') in type II diabetes (Wing *et al*, 1994; Kelley *et al*, 1993; Christiansen *et al*, 1995).

(iii) The liver is therefore responsible for whole-body fuel selection in the post-absorptive state. The rate of release of glucose into the circulation, and the secondary consequences of this, particularly the alterations in insulin secretion, profoundly affect the fuel mixture selected by tissues. Implications for the role of the liver in the etiology of obesity have only recently been considered. Pagliassotti *et al* (1997a,b) have shown that non-suppressability of HGP by insulin is the best predictor of obesity in rats fed *ad libitum* high-fat diets. Over-expression of the GNG enzyme PEP-CK in the liver of transgenic mice (Valera *et al*, 1994) and rats (Rosella *et al*, 1995) results in hyperinsulinemia, obesity and ultimately hyperglycemia. Therefore, a liver enzyme can be responsible for obesity and its sequelae. It is of interest to note that hepatic insulin can be predicted to exert opposite effects on body composition than muscle insulin resistance exerts (Swinburn *et al*, 1991). The former is characterized by increased HGP and peripheral glucose utilization (a high-flux state for glucose), with reduced fat oxidation and increased CHO oxidation in the whole body; the latter by reduced glucose utilization and production (a low-flux state for glucose), with reduced CHO oxidation and increased fat oxidation in the whole body (Swinburn *et al*, 1991). Accordingly, the presence of hepatic insulin resistance out of proportion to muscle insulin resistance will tend to *increase* body fat stores, whereas muscle insulin resistance disproportionate to liver will tend to *reduce* body fat.

In this context, it is also apparent that that the liver is more likely to contribute to obesity by overproducing glucose than by converting CHO to fat. Though counterintuitive on the surface, this conclusion follows from the basic physiologic premises just discussed and has important implications for genetic and pathophysiologic investigations of the etiology of obesity.

(iv) Finally, the absence of an analogous mechanism for matching fuel selection to changes in fat intake should be noted (Figure 7). Thus, low fat diets *per se* do not cause changes in HGP or help drive RQ to match FQ; instead, it is the reciprocal change in CHO intake that appears to provide the signals that alter fuel selection, including tissue oxidation of fat. Sidossis & Wolfe (1996) have recently termed this 'the Randle cycle reversed', namely, that CHO availability controls tissue fat oxidation more than FFA availability controls tissue CHO oxidation, if both substrates are present concurrently. Metabolic mechanisms to explain regulation of tissue fat oxidation by CHO availability are well described (McGarry & Foster, 1980). The absence of specific mechanisms to match fat oxidation to increased fat intake (Figure 7) contribute to sensitivity to dietary fat-induced obesity, (Pagliassotti *et al*, 1997a,b).

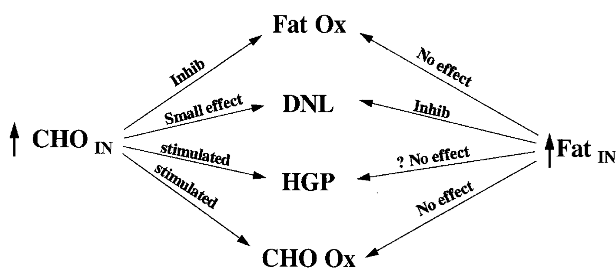


Figure 7 Proposed metabolic response to surplus dietary CHO or fat. Effects of increased CHO intake are shown from the left; effects of increased fat intake from the right. Abbreviations are as in the text. Inhib, inhibited.

Unanswered questions concerning the regulation and functions of DNL

There are still many unanswered questions concerning the regulation of DNL and its role in human physiology or disease.

(1) What constrains DNL under usual dietary conditions? Is there a signal that restrains DNL in liver or adipose tissue, despite the availability of substrate, enzymes and cofactors and the inducibility of lipogenic gene expression? Studies in animals and *in vitro* suggest that specific FAs exert negative feedback on expression of DNL genes and enzymes (Girard *et al*, 1994; Sul *et al*, 1993). Translation of these findings to physiology is not obvious, however. How much fat, and in what tissue, feeds back on the DNL pathway?

Results with a transgenic/knockout mouse model of dietary fat malabsorption may be informative in this regard. Young *et al* have developed a mouse with the endogenous apoB gene knocked out and a human apoB transgene expressed in liver only (Young *et al*, 1995). Accordingly, these mice are unable to form chylomicrons or efficiently absorb dietary fat; the result is that CHO represents a high percent of absorbed nutrients. Despite the marked reduction of dietary fat absorption (estimated to be 10–20% of normal, based on serum concentrations of lipid-soluble vitamins), these animals maintain normal plasma concentration of triglycerides and cholesterol and are able to accumulate body fat, though at a much lower rate than in normal mice (Young *et al*, 1995). These observations suggested that DNL might occur at a very high rate, to maintain hepatic lipoprotein secretion and adipose fat deposition. To address this question, we have measured DNL, FFA flux and re-esterification to hepatic TG in these mice (Turner *et al*, 1998). The most striking result was a greatly accelerated incorporation of plasma ^{13}C-FFA into hepatic and VLDL-TG (that is, accelerated re-esterification of plasma FFA). Hepatic fractional DNL was only slightly elevated (15% *vs* 5%) while adipose DNL appeared to be identical or even lower after several weeks of oral ^{13}C-acetate feeding in chylomicron-deficient mice compared to normal mice.

We (Turner *et al*, 1998) concluded from these results that very low dietary fat absorption does not induce DNL in mice; and that hepatic DNL may be inhibited by the presence of FA in adipose stores even in small quantities,

by virtue of the capacity of the liver to avidly take up and re-esterify adipose-derived FFA. Thus, FA in adipose stores may inhibit DNL in liver. If true, a prediction is that lipolysis inhibitors might markedly disinhibit hepatic DNL in these mice or on low-fat diets in general.

Another approach to the question of factors limiting DNL is by comparison of different substrates. The liver may simply have a limited capacity for oxidation of pyruvate derived from glycolysis to acetyl-CoA. Results with EtOH (Figure 2) do not support this explanation, however (Siler *et al*, 1996): although EtOH enters the liver at the 2-carbon level, the quantitatively modest hepatic lipogenesis and the release of large amounts of free acetate after EtOH ingestion suggests either limited capacity to convert acetate to acetyl-CoA or a block in conversion of acetyl-CoA to fat (that is, intrinsically limited DNL capacity), with subsequent acetyl-CoA deacylation.

What is the contribution of adipose DNL in humans? Although most work has focused on hepatic DNL in humans and enzyme levels have suggested adipose to be a weakly lipogenic tissue in people (Shrago *et al*, 1971; Weiss *et al*, 1986; Sjostrom, 1973), recent evidence (Aarsland *et al*, 1996) suggests that adipose tissue may be the place to look for quantitatively significant DNL during overfeeding, particularly by the parenteral route. Before adipose DNL can be measured *in vivo*, however, two technical problems will need to be solved. Firstly, the huge adipose TG pool size results in such large dilution of newly synthesized TG that reliable measurement of label incorporation has not been possible in human subjects. One solution might be to carry out much longer isotopic studies; another idea is to isolate diglycerides and/or monoglycerides from adipose (since these are believed to be either en route to or from TG). The second problem is how to exclude the possible contribution from hepatic-synthesized TG that were transported to adipose tissue. Neither technical problem has yet been solved.

It should be pointed out here that quantitatively significant *hepatic* DNL is not a physiologic impossibility. If measurements *in vivo* under lipogenic conditions showed the following values, quantitatively significant DNL would be present: fractional DNL contribution 75–80% of non-essential FA in VLDL-TG, non-essential FA comprising 75–80% of TG, and total VLDL-TG production and clearance rates 4- or 5-fold elevated (to 100–125 g/d). Hepatic DNL would then contribute 60–75 g new fat synthesis daily. Hepatic DNL rates of this magnitude would have a major impact on macronutrient balances, but have not been found.

(3) Are there any inborn errors of metabolism leading to elevated rates of DNL? At least two genetic conditions might be associated with greatly increased rates of DNL: one found in nature, one induced by recombinant DNA techniques. Glycogen storage disease-type I (GSD-1) is a relatively common inborn error of metabolism due to the absence of hepatic glucose-6-phosphatase activity (Chen & Burchell, 1994). Hepatic glycogenosis and fat accumulation, elevated plasma lactate concentrations, marked hypertriglyceridemia, absence of serum ketones and borderline-hypoglycemia are included in the phenotype of GSD-1. A role for hepatic DNL in the TG accumulation in liver and plasma has been proposed and might be predicted from the extremely high availability of 3-carbon (lactate/pyruvate) and glucose (glycogen) precursors in the liver. DNL has not yet been directly measured in GSD-1, however.

Shimano *et al* (1996) over-expressed the steroid response element-binding protein (SRE-BP) in transgenic mice. SRE-BP stimulates transcription and expression of cholesterogenic and DNL genes. Mice over-expressing SRE-BP exhibited markedly elevated serum TG and cholesterol concentrations and fatty livers, with evidence of increased DNL (Shimano *et al*, 1996), although the quantitative contribution from DNL was not assessed and the animals did not become obese. Whether an analogous genetic disorder exists in humans has not been determined.

(4) What about embryonic (fetal) and/or mammary gland DNL? One possible explanation for why the enzymatic machinery for DNL is present but used so sparingly in adult humans is that this represents a vestigial pathway in post-natal life, that is, the predominant function of DNL occurs *in utero*. The rationale behind this hypothesis is that during the third trimester in particular the human fetus has a very large demand for lipids, for deposition of subcutaneous fat as well as for myelination of the developing central nervous system. The brain is 60% fat by wet weight and represents 20% of body weight in the newborn human (360 g fat in the brain of a 3 kg infant). If body fat is 20% of body weight, in addition (600 g) close to 1 kg FA needs to be deposited, mainly during the third trimester. The fetus therefore needs upwards of 10–12 g FA/d for storage purposes alone. Transport of lipoproteins or FFA across the placenta from the mother is inefficient, however, and appears inadequate to meet the lipid requirements of the developing fetus. A role for greatly elevated rates of fetal lipogenesis might therefore be predicted. Fetal DNL has not been directly measured in humans. Some indirect evidence for high DNL *in utero* comes from studies of premature infants, who often exhibit extraordinarily high values of NP RQ (for example, >1.20, Dr. W. Chwals, personal communication) in response to parenteral nutrition even with intralipid (triglyceride emulsions) included. If these premature infants are still manifesting *in utero* physiology, it might be inferred that DNL is indeed elevated *in utero*. This hypothesis remains unconfirmed.

Milk secreted by the mammary gland is high in fat. Although the source of this fat in human breast milk has not been directly measured, lactating mammary gland expresses DNL enzymes at high levels. Mammary gland DNL represents a potential role for this pathway, but this has not been yet proven.

(5) Are there other regulatory functions of DNL in tissues? The first committed step in the lipogenic pathway is synthesis of malonyl-CoA from acetyl-CoA and carbon dioxide, catalyzed by the enzyme acetyl-CoA carboxylase (Bloch, 1977; Girard *et al*, 1994). Malonyl-CoA inhibits transport of FA-CoAs into mitochondria by inhibiting carnitine acyl-transferase I activity (McGarry & Foster, 1980). Therefore, within a given cell, the occurrence of lipogenesis is believed generally to be inconsistent with β-oxidation of fatty acids and to promote cytosolic esterification of FA-CoAs.

This indirect function of the lipogenesis pathway has been proposed to play a regulatory role in several tissues. In liver, malonyl-CoA has anti-ketogenic and pro-esterifying effects on FA-CoAs, thereby promoting TG synthesis and assembly of ApoB containing particles. In muscle, malonyl-CoA may serve as an important signal regulating fuel selection (despite the lack of recognized lipogenic fate for malonyl-CoA in muscle) (Ruderman & Herrera, 1968; Ruderman *et al*, 1969; Ruderman & Haudenschild, 1984). In pancreas, Prentki *et al* (1992) have proposed that malonyl-CoA levels increase in response to β-cell glucose utilization, leading to an accumulation of FA-CoAs in the cell cytosol, which in turn serves as a metabolic signal for insulin secretion. Components of the DNL pathway may therefore have important regulatory functions in tissues that are not considered to be classically lipogenic.

Another possible regulatory function of DNL is the synthesis of fatty acids, such as myristate, that are used for acylation of proteins (Johnson *et al*, 1994). Whether the myristate used for myristoylation is derived from diet or DNL is unknown, at present. If the source is diet, then myristate should be considered an essential FA. More likely, these FAs are synthesized *de novo* from acetyl-CoA, in which case DNL might be regulated for reasons other than the needs of the energy economy alone.

(6) What would be the physiologic consequences if DNL were a more active pathway? It is interesting to speculate what would happen metabolically if DNL in fact served as the disposal route for transient surpluses of CHO. One physiologic model of this situation, wherein the DNL pathway is wide-open might be the marmot prior to hibernation (Young & Sims, 1977). These animals deposit large quantities of body fat in preparation for hibernation and DNL has been reported to be markedly elevated. Might DNL play an important role in this setting of (adaptive) positive fat balance? According to the model of Flatt (1987), carbohydrate stores are postulated to influence food intake and satiety. Evidence has been presented in support of a role for liver glycogen (or other metabolites related to hepatic hexoses) in appetite regulation (Sullivan *et al*, 1974; Russek, 1963, 1981; Russek & Stevensen, 1972), although the hypothesis remains largely unproven. The occurrence of a rapid flux through DNL from CHO in pre-hibernation animals might keep tissue glycogen stores relatively low, prevent satiety and promote continued food intake, in addition to directly contributing fat to body stores. It should be noted that tissue fuel selection is *not* matched to food intake under these conditions (by intent), unlike during most of an adult animal's life.

If pre-hibernating mammals indeed exhibit wide-open flow through the DNL pathway, some predictions (see above) might be testable. One need *not* postulate increased HGP or reduced tissue fat oxidation in the post-absorptive state in these animals, for example, despite a marked excess of CHO intake, if surplus CHO is removed from tissue stores by conversion to fat during the absorptive phase. Another way to test the functional consequences of removing normally constrained DNL might be through transgenic techniques (that is, over-expressing the enzymes of DNL and/or pyruvate oxidation in liver or adipose tissue).

Table 9 What are the physiologic functions of *de novo* lipogenesis?—Speculations

(1) There are no functions (vestigial pathway in humans)
(2) Necessary in embryonic development—for CNS lipid synthesis (vestigial pathway in post-natal life)
(3) Important on low-fat diets—suppressed by un-natural modern diet (vestigial pathway culturally)
(4) Only important after long-term surplus CHO (glycogen overflow pathway)
(5) Serves signal or regulatory functions (e.g. antiketogenesis, tissue fat oxidation, insulin synthesis, etc.)

(7) What are the physiologic functions of DNL? This discussion raises the larger question: What are the physiologic functions of DNL? A number of possibilities can be considered (Table 9). Most of these have been discussed above, but no definitive answer to the question can be provided at this time. Differentiating between these possibilities will have important implications for a number of questions in metabolic regulation and the control of energy balance.

(8) Does DNL contribute to human disease? Even with relatively low quantitative fluxes through the pathway, DNL may be involved in a variety of diseases (Table 10). FA synthesized through DNL in the liver appear to be preferentially secreted as VLDL-TG rather than stored in the cytosolic TG pool (Gibbons, 1990; Gibbons & Burnham, 1991). If hepatic FA from DNL are more effective at protecting apoB from proteolysis and promoting VLDL-particle assembly than FA derived from plasma FFA or lipoprotein remnants (Gibbons, 1990; Gibbons & Burnham, 1991), then hyperlipidemia associated with stimulators of DNL (EtOH, fructose, cytokines) might reflect effects on lipoprotein assembly out of proportion to its quantitative contribution of new FAs. Fatty liver, the precursor to fibrosis in alcoholic cirrhosis, might also be related to DNL (Siler *et al*, 1996). Hepatic fat deposition in GSD-1 may contribute to the tendency for liver tumors (Chen & Burchell, 1994) and, as discussed above, these FA could derive in part from the DNL pathway. Another result of elevated rates of DNL is a change in FA composition of VLDL-TG to a more saturated profile (Hudgins *et al*, 1996). If membrane FA composition in certain cells throughout the body also become more saturated as a result, a variety of physiologic consequences might result, including insulin resistance (Storlein *et al*, 1996). Finally, alterations in intracellular signaling pathways related to changes in myristolylation, palmitoylation, malonyl-CoA availability, etc. (McGarry & Foster, 1980; Prentki *et al*, 1992; Johnson *et al*, 1994) could be involved in disorders

Table 10 Possible contribution of hepatic lipid synthesis to human disease

(I) Plasma lipids and lipoproteins (atherogenesis)
(II) Energy balance (obesity, insulin resistance)
(III) Bile synthesis (cholelithiasis, atherosclerosis)
(IV) Hepatic fat (fatty liver, cirrhosis)
(V) Cell membrane FA composition (physiologic consequences)
(VI) Intracellular signaling (myristoylation, malonyl-CoA, etc.)

related to these mediators. These potential roles of DNL in disease are speculative at present, but deserve investigation.

Summary

DNL is not the pathway of first resort for added dietary CHO in humans, at least on Western (high-fat) diets. DNL *can* occur, but it generally does not. A 'functional block' therefore exists between CHO and fat in humans, analogous to the absolute biochemical block in the direction from fat to carbohydrate in all animals. Therefore, the two major macronutrient energy sources are not interconvertible currencies in the mammalian organism; they must be considered separately and are probably regulated independently, by separate signals and toward separate ends. The major insight concerning DNL is therefore a negative one. Many questions related to this central observation still remain unanswered: what is the functional significance of DNL in adult life (Table 9)? What are the ultimate limits of DNL in humans? Is DNL only used as a final 'safety-valve' for CHO in the organism? What constrains DNL in human lipogenic tissues? Are there regulatory roles played by DNL that we have not yet identified? Regardless of the answers to these questions, the metabolic and clinical consequences of the apparent functional block between CHO and fat are profound and have only begun to be understood.

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