J Comp Physiol B (2011) 181:1–17
DOI 10.1007/s00360-010-0528-0

REVIEW

Hypercarnivory and the brain: protein requirements of cats reconsidered

Regina Eisert

Received: 26 August 2010 / Revised: 19 October 2010 / Accepted: 25 October 2010 / Published online: 19 November 2010

Abstract The domestic hypercarnivores cat and mink have a higher protein requirement than other domestic mammals. This has been attributed to adaptation to a hypercarnivorous diet and subsequent loss of the ability to downregulate amino acid catabolism. A quantitative analysis of brain glucose requirements reveals that in cats on their natural diet, a significant proportion of protein must be diverted into gluconeogenesis to supply the brain. According to the model presented here, the high protein requirement of the domestic cat is the result of routing of amino acids into gluconeogenesis to supply the needs of the brain and other glucose-requiring tissues, resulting in oxidation of amino acid in excess of the rate predicted for a non-hypercarnivorous mammal of the same size. Thus, cats and other small hypercarnivores do not have a high protein requirement per se, but a high endogenous glucose demand that is met by obligatory amino acid-based gluconeogenesis. It is predicted that for hypercarnivorous mammals with the same degree of encephalisation, endogenous nitrogen losses increase with decreasing metabolic mass as a result of the allometric relationships of brain mass and brain metabolic rate with body mass, possibly imposing a lower limit for body mass in hypercarnivorous mammals.

Keywords Hypercarnivores · Allometry · Brain metabolism

Communicated by I.D. Hume.

R. Eisert (✉)
Smithsonian Environmental Research Center,
Edgewater, USA
e-mail: eisertr@si.edu

R. Eisert
Gateway Antarctica, University of Canterbury,
Christchurch, New Zealand

Introduction

The domestic cat (*Felis silvestris*) is an example of a 'hypercarnivore', i.e. an animal that has evolved to consume vertebrate prey almost exclusively (Holliday and Steppan 2004; Wang and Tedford 2008). Hypercarnivores such as cats and mink (*Mustela vison*) have a higher dietary protein requirement than omnivorous mammals (Greaves and Scott 1960; Rogers and Morris 1979; NRC 1982; MacDonald et al. 1984; Damgaard et al. 1998; NRC 2006), high endogenous nitrogen losses (Table 1), high in vitro activities of enzymes involved in protein catabolism (Rogers et al. 1977; Sørensen et al. 1995), and appear to have a limited ability to adjust protein oxidation to low dietary intakes of protein (Tarttelin 1991; Damgaard et al. 1998; Rogers and Morris 2002). The specific metabolic pattern of the cat appears to be preserved even though cats used for scientific research are generally maintained on diets that are much higher in carbohydrate, and frequently lower in protein, than their ancestral prey-based diet. The current paradigm, based on the work of Rogers, Morris and others on cats, states that the high protein requirement of cats is due to an inability to downregulate enzymes that catabolise amino acids, resulting in a failure to conserve amino nitrogen at low protein intakes (Rogers et al. 1977; Rogers and Morris 2002). Thus, the inability of cats to downregulate protein catabolism is conceptualised as equivalent to other functional losses associated with hypercarnivory in cats, such as the inability to synthesise sufficient taurine, or to convert β-carotene to vitamin A.

However, animals in general have a limited capacity to store nitrogenous compounds, necessitating tight synchronisation between the intake and catabolism of protein (Millward 1995; Russell et al. 2000), and this also applies to cats. Cats can adjust protein oxidation to match a range

Table 1 Comparison of endogenous urinary nitrogen loss (EUNL), obligatory nitrogen loss (ONL), and fasting urinary nitrogen loss (FUNL) in a range of mammals

Species	EUNL	ONL	FUNL
Rat (*Rattus norvegicus*)	116[a]–128[b]	170[c]–273[d]	380[e]–436[d]
Rabbit (*Oryctolagus cuniculus*)	–	–	380[f]
Mink (*Mustela vison*)	280[g]	–	586[h,*]
Cat (*Felis silvestris*)	360[b,i]	–	283[j]
Dog (*Canis lupus*)	199[k]	263[k,†]	360[c]–431[l]
Human (*Homo sapiens*)	99[m]–108[n]	140[m,o]–150[p]	240[q]–416[r]
Pig (*Sus scrofa*)	163[b,‡]	–	194[l]–240 [s]
Sheep (*Ovis aries*)	–	–	350[t]
Grey seal pup (*Halichoerus grypus*)	–	–	180[u,‡]
Marmoset (*Callithrix jacchus*)	62.1[v]	–	–
Predicted mammalian average	140[w]	272[p]	418[p]

Values are in mg N $kg^{-0.75}$ day^{-1}

* Short-term fast, † urinary plus faecal nitrogen loss, ‡ immature animals

[a] Uezu et al. (1983); [b] Hendriks et al. (1997); [c] Howe et al. (1912); [d] Henry et al. (1987); [e] Goodman et al. (1984), day 3 of starvation; [f] Hannaford et al. (1982); [g] Tauson et al. (2001); [h] Tauson et al. (1997); [i] Miller and Allison (1958); [j] Biourge et al. (1994), average of weeks 2–6; [k] Kendall et al. (1982), recalculated mean for animals that maintained body mass only; [l] Lusk (1928); [m] Calloway and Margen (1971); [n] Scrimshaw et al. (1972); [o] Rand et al. (2003); [p] Henry and Collingwood (1998); [q] Owen et al. 1998, obese, average of day 2–18 of fast; [r] Göschke et al. (1975), lean men and women, day 6 of starvation; [s] Müller et al. (1984), 21-kg minipigs, day 4 of starvation; [t] Blaxter (1962); [u] Nordøy et al. (1990); [v] Flurer et al. (1988); [w] Smith (1926)

of dietary protein concentrations provided their diet contains at least 14–20% of metabolisable energy (ME) intake as protein (Russell et al. 2002, 2003; Green et al. 2008; Wester et al. 2008). This is considerably higher than the minimum of 6–8% of ME required by humans, rats and dogs for maintenance (Rogers and Morris 2002; Rand et al. 2003; NRC 2006; Green et al. 2008). But exactly why cats cannot adjust the catabolism of amino acids to lower intakes of protein sufficient for other species is not clear. According to the general model of nitrogen catabolism in mammals, the lower limit of amino acid catabolism an animal can adapt to is dictated by the rate of whole-body protein turnover and the concomitant obligatory nitrogen loss (Waterlow 1999). Yet, protein turnover in cats is only one-half to one-third of rates measured in other mammals (Russell et al. 2003), and thus cannot explain why cats need to catabolise amino acids at the high rates observed. In the words of Russell et al. (2003), "*the apparently high feline protein requirement remains unexplained and is probably not a simple reflection of an inability to adapt hepatic catabolic capacity to variation in protein intake*".

Previous work on the protein requirements of cats has established the capacity of cats for handling large protein loads and their failure to sufficiently reduce catabolism when faced with low-protein diets (Rogers et al. 1977; Russell et al. 2000, 2002, 2003; Rogers and Morris 2002; Russell 2002; Green et al. 2008). In this paper, I propose a mechanistic explanation for why cats are unable to down-regulate catabolic enzymes, and thus amino acid oxidation, to the same degree as non-hypercarnivorous mammals. Cats have a relatively large brain and a high metabolic demand for glucose that must be met by amino acid-based gluconeogenesis when the cat is consuming a prey-based diet. I propose that gluconeogenesis from amino acids in cats is constitutive and represents a significant metabolic sink for amino acids that increases the minimum protein requirements of cats above that of non-hypercarnivorous mammals. The proposed explanation rests on three hypotheses:

Hypothesis 1: The cat is a small, hypercarnivorous mammal with a relatively large brain and hence a large endogenous glucose demand. The cat has evolved specific metabolic strategies to meet its endogenous glucose demand while consuming a very low-carbohydrate diet without resorting to hyperketonaemia.

Hypothesis 2: In cats, amino acids are channelled into gluconeogenesis at a rate sufficient to meet the endogenous glucose demand of brain and other glucose-dependent tissues, independent of dietary carbohydrate intake (*obligatory gluconeogenesis*).

Hypothesis 3: Because cats have a relatively high endogenous glucose demand, obligatory gluconeogenesis results in high rates of amino acid oxidation and endogenous nitrogen losses that exceed the rate of endogenous nitrogen loss predicted for a cat-sized, non-hypercarnivorous mammal. Since the endogenous nitrogen loss determines the

lower limit for protein requirements, obligatory gluconeogenesis thus increases minimum protein requirements of cats relative to non-hypercarnivorous mammals.

The main premises underlying these hypotheses will be discussed in detail and placed in a comparative mammalian context. The proposed alternative explanation for the high protein requirement of the domestic cat also addresses several as-yet unresolved questions, such as why the fasting urinary nitrogen loss (FUNL) of cats should be lower than their endogenous urinary nitrogen loss (EUNL), or why domestic cats may be particularly prone to developing obesity, insulin resistance and type-II diabetes mellitus.

What is the natural diet of the cat?

Adult, non-reproductive cats have no demonstrable dietary requirement for carbohydrate (MacDonald et al. 1984), but like all mammals, cats have certain tissues and organs that absolutely require glucose. Examples include brain, spinal cord and peripheral nerves, red blood cells, renal medulla, testes, mammary gland during lactation, and the pregnant uterus (Waites and Setchell 1964; Sokoloff et al. 1977; Oftedal et al. 1993; Berg et al. 2002; Sunehag et al. 2002). Metabolic dependence on glucose in cats is illustrated by the fact that feeding behaviour can be triggered if plasma glucose is lowered by administration of insulin or 2-deoxyglucose (Rowland 1981). Mammalian body stores of carbohydrate—glycogen in liver and muscle—are quite limited, and account for only 200–500 g total in humans (Flatt 1995) and ca. 0.65% of fat-free mass in rats (Even et al. 2001; Morifuji et al. 2005). Since glucose is an essential substrate for the cat, the question is how this demand can be met on a hypercarnivorous diet. As shown in Table 2, a cat consuming small vertebrate prey would receive only 1–2% of its ME intake as carbohydrate, unless ingesting selectively only body parts that are high in glycogen such as liver (Table 2). Even a mouse fully

Table 2 Composition of potential prey items, human-provided foods, and dietary recommendations for cats

Food item	BM (g)	Fat	Protein	Water (% BM)	Ash	CHO	ME (kJ g^{-1})	Fat	Protein (% ME)	CHO
Vertebrate prey										
Old-field mouse[a] (*Peromyscus polionotus*)	11.4	12.0	17.0	64.7	3.67	0.57	7.46	61	38	1.3
Common vole[b] (*Microtus arvalis*)	16.1	14.3	16.2	63.5	3.30	0.56	8.18	66	33	1.1
Bank vole[c] (*Clethrionomys glareolus*)	21.3	4.00	20.5	71.2	3.70	0.62	5.04	30	68	2.1
Lab mouse[d] (*Mus musculus*)	20.5	14.5	18.1	62.7	3.65	0.56	8.57	64	35	1.1
Lab mouse, after a meal of wheat*	22.0	14.3	17.9	61.8	3.60	1.61	8.63	62	35	3.1
Rabbit, newborn[e] (*Oryctolagus cuniculus*)	53.0	5.75	11.6	79.9	2.20	0.61	4.21	52	46	2.4
1-Day chick[f] (*Gallus domesticus*)	4.5	5.73	16.6	74.4	1.16	0.46	5.02	43	55	1.6
Small passerine bird[f,g,h,i,j]	10–100	5–15	19–21	63–71	~3.2	0.4–0.5	5.5–8.9	35–64	35–64	1–2
Human-provided foods										
Whole milk[k]	–	–	–	–	–	–	2.51	49	21	30
Beef liver, fresh[k]	–	–	–	–	–	–	5.64	25	63	12
Dry kibble 1[l]	–	–	–	–	–	–	13.5	23	40	37
Dry kibble 2[m]	–	–	–	–	–	–	20.5	50	33	18
Canned wet 1[n]	–	–	–	–	–	–	5.49	53	29	18
Canned wet 2[o]	–	–	–	–	–	–	4.46	46	38	16
Experimental 'high-protein' diet[p]	–	–	–	–	–	–	–	39	50	11
NRC recommendation for maintenance[q]	–	–	–	–	–	–	–	20	20	–

Carbohydrate (CHO) content of prey was estimated assuming that fat-free mass of rodents contains 0.65% and of birds, 0.50% carbohydrate (Swain 1992; Edwards et al. 1999; Even et al. 2001; Morifuji et al. 2005). Metabolisable energy (ME) was calculated from Atwater factors for protein, carbohydrate, and fat of 16.74, 16.74, and 37.66 MJ kg^{-1} (NRC 2006). Note that foods may vary in digestibility and true ME values may differ from calculated values

BM body mass

* See Appendix for calculations

[a] Kaufman and Kaufman (1977); [b] Sawicka-Kapusta (1970); [c] Sawicka-Kapusta (1974); [d] Bailey et al. (1960); [e] Xiccato et al. (1999); [f] Dierenfeld et al. (2001); [g] Blem (1976); [h] Swain (1992); [i] Taruscio and Murphy (1995); [j] Edwards et al. (1999); [k] USDA National Nutrient Database (2009); [l] 'Hills Science diet for cats, adult indoor', information provided on manufacturer's website, Hill's Pet Nutrition, Inc. (2010); [m] 'Eukanuba feline recovery dry', Martin and Rand (1999); [n] 'Whiskas selected protein', Martin and Rand (1999); [o] 'Iams premium pate with tender chicken and liver', information provided on manufacturer's website, Iams products for cats (2010); [p] Green et al. (2008); [q] NRC (2006)

'gut-loaded' with digestible carbohydrate (e.g. wheat grain) contains less than 4% of ME as carbohydrate (Table 2; Appendix), but secondary consumption of carbohydrate is unlikely to be of practical significance as cats generally do not consume the digestive tracts of their prey (Leyhausen 1979). To put macronutrient intake by cats into perspective, a prey-based diet contains 1–2:30–68:30–68% of ME as carbohydrate:fat:protein (Table 2), compared with typical macronutrient ratios of ca. 15–35:20–50:30–40 in commercial cat food (Martin and Rand 1999; Table 2), 55:30:15 in human reference diets (Gannon and Nuttall 2004), 20:50:30 in human low-carbohydrate, high-protein diets (Gannon and Nuttall 2004), and 8:50:42 for the traditional diet of the Greenland Inuit ("Eskimo"; Heinbecker 1928). Assuming a daily ME consumption of 1,000 kJ or 6–12 small rodents (NRC 2006; Table 2), a cat would receive between 0.6 and 1.1 g of carbohydrate from its diet. At a fat content of 12% for wild prey, the cat would ingest an additional 1.8 g of glycerol supplying a maximum of 1.8 g glucose contingent on complete oxidation of ingested fatty acids (Cahill et al. 1966; Streja et al. 1977; MacDonald et al. 1984). Thus, the amount of carbohydrate typically available from a prey-based diet (range 2.4–2.9 g day^{-1}) is insufficient to meet the net glucose demand of the brain (3.1 g day^{-1}; Table 4), even without considering glucose use by other obligate glucose-consuming tissues. No data are available for cats, but glucose demand by obligate glucose-consuming tissues other than brain accounts for ca. 40% of brain glucose consumption in humans (Cahill et al. 1966; Sokoloff et al. 1977), and it is possible that the proportion of glucose use by extracerebral tissues is greater in cats as a result of their relatively smaller brain (Table 4).

It appears that a prey-based diet routinely supplies insufficient carbohydrate to meet metabolic demands in the hypercarnivorous cat. In contrast, omnivores and herbivores are likely to encounter carbohydrate deficiency only sporadically and most likely as a result of starvation or in the context of physiological states that cause an increase in endogenous glucose demand. As discussed in the following sections, omnivores and hypercarnivores differ in their response to dietary carbohydrate insufficiency: in omnivores, glucose is partially replaced by ketone bodies, whereas high rates of gluconeogenesis remove any dependence on dietary carbohydrate in hypercarnivores.

Ketone bodies: glucose substitutes

Ketone bodies (acetoacetate, β-hydroxybutyrate, and acetone) are lipid-derived metabolites that provide a substitute fuel that can be taken up and utilised by most tissues including heart and brain (Owen et al. 1967; Robinson and Williamson 1980). Conditions that stimulate ketogenesis are a low insulin:glucagon ratio, high rates of lipolysis and fatty acid oxidation, and reduced intracellular availability of glucose, as is the case in starvation, on carbohydrate-deficient diets, or in insulin-dependent diabetes mellitus (Klein and Wolfe 1992; Mitchell et al. 1995). In general, omnivorous mammals respond to both carbohydrate deficiency and total starvation with an increase in ketone body production, although the degree of hyperketonaemia may differ considerably between species (Robinson and Williamson 1980). Both humans and rats respond to low-carbohydrate diets with rapidly developing hyperketonaemia (Likhodii et al. 2002; Astrup et al. 2004). The ketogenic response is exacerbated if endogenous glucose demands are increased, e.g. during pregnancy and lactation (Felig 1973; Doreau et al. 1981; Jeanblain and Durix 1985; Mitchell et al. 1995; Dalrymple 2004; Ayala et al. 2006). Domestic ruminants, which rely on gluconeogenesis rather than dietary carbohydrate to meet their glucose needs, appear particularly susceptible to pathological hyperketonemia under conditions of elevated glucose demand (Heitmann et al. 1987). Simply put, the elevation of ketone bodies above basal levels signals the carbohydrate-deficient state; conversely, low circulating concentrations of ketone bodies indicate that the rate of glucose appearance (dietary plus endogenous) is sufficient to meet metabolic glucose demands. Ketone bodies are utilised by brain proportional to their arterial concentrations (Hawkins et al. 1971; Robinson and Williamson 1980; Blomqvist et al. 2002) and in humans, can provide as much as 60% of brain substrate requirements during prolonged starvation (Cahill 1983). This partial substitution of glucose by ketones not only helps to maintain fuel supply to the brain, but also reduces the need for protein catabolism to provide gluconeogenic precursors (Cahill 1983; VanItallie and Nufert 2003) and thus greatly prolongs survival time, as mammals tend to have a greater capacity for catabolism of body fat than body protein. However, brain tissue cannot run on ketone bodies as an exclusive substrate and continues to use glucose at all times (Sokoloff et al. 1977; Hertz and Dienel 2002).

Adult cats fed very low-carbohydrate diets ($\leq$10% of ME) do not develop hyperketonaemia (Chugani et al. 1991; Dobenecker et al. 1998; Pazak et al. 1998; Blanchard et al. 2002; Thiess et al. 2004). Nevertheless, cats develop significant hyperketonaemia during starvation (Blanchard et al. 2002) and in insulin-deficient diabetes (Stadie et al. 1940), indicating that lack of ketone body accumulation in cats on very low-carbohydrate diets is not due to a defect in ketogenesis. This metabolic pattern in cats is consistent with high rates of gluconeogenesis sufficient to suppress ketogenesis in the fed state, and reduction of gluconeogenesis and gradual substitution of glucose by ketone bodies to spare protein during prolonged starvation, as

indicated by a reduction in daily nitrogen loss (FUNL < EUNL; Table 1). Cats likely evolved under conditions of intermittent deprivation, e.g. due to seasonal changes in abundance and composition of prey, and retain the ability to utilise hyperketonaemia as an adaptive response during total starvation.

Gluconeogenesis

A number of authors have proposed that cats have constitutively high rates of gluconeogenesis and in this regard resemble ruminant mammals (Rogers et al. 1977; Kettelhut et al. 1980; MacDonald et al. 1984; Lobley 1992). This is particularly remarkable since most cats used in scientific studies are maintained on diets (Miller and Allison 1958; Greaves and Scott 1960; Biourge et al. 1994; Hendriks et al. 1997; Russell et al. 2000, 2002, 2003; Green et al. 2008) that contribute 5–20 times the amount of carbohydrate on a ME basis than what might be considered the 'natural' diet of the species (Table 2). In other words, high rates of gluconeogenesis in the cat appear to be essentially preserved despite a significant intake of carbohydrate. Because postprandial gluconeogenesis has not been measured in cats, it is currently not possible to assess to what extent, if any, gluconeogenesis is reduced following the consumption of carbohydrate. In humans and rats, ingestion of a mixed meal suppresses hepatic glucose output by insulin-mediated mechanisms as well as by the direct effects of postprandial hyperglycaemia (Tonelli et al. 2005). Insulin suppresses hepatic glucose output directly by inhibiting glycogenolysis and gluconeogenesis, and indirectly by inhibiting lipolysis and stimulating lipogenesis, thus reducing the availability of non-esterified fatty acids (Sugden 2007). Insulin resistance and incomplete suppression of gluconeogenesis in response to insulin result in reduced glucose tolerance, i.e. an increase in the time required to restore baseline plasma glucose after carbohydrate administration as part of a glucose tolerance test. Glucose tolerance in cats is low compared with dogs, humans, rats and horses (Kaiyala et al. 1999; Appleton et al. 2001; Hoffman et al. 2003; Morens et al. 2006), consistent with the failure to suppress gluconeogenesis in the presence of excess carbohydrate. A similar pattern of glucose intolerance, also known as 'starvation diabetes' (Lundbæk 1948), can be induced in omnivorous species by adaptation to a high-protein, carbohydrate-free diet (McClellan and Du Bois 1930; Belo et al. 1976) or by fasting (Féry et al. 1990; Horton and Hill 2001). When a glucose load is given to normal rats, hepatic glucose production is completely suppressed 1 h after dosing, whereas there is no detectable suppression of hepatic glucose production in starved rats under the same conditions (Smadja et al. 1990). The crucial difference between cats on the one hand and humans and rats on the other is while the latter can be habituated to a high-protein, very low carbohydrate diet and develop similar metabolic patterns to cats (high rates of protein-based gluconeogenesis, glucose intolerance), they revert back to their normal omnivorous pattern (low and variable gluconeogenesis, high glucose tolerance) when returned to a mixed diet.

Assuming that glucose tolerance in cats is reduced as a result of constitutively high rates of gluconeogenesis, it might be predicted that cats are susceptible to developing obesity and insulin resistance on diets containing high levels of carbohydrate. Clinical observations confirm that in cats, consumption of a diet containing a significant proportion of ME as available carbohydrate may be associated with hyperglycaemia, hyperinsulinaemia, insulin resistance and obesity, whereas isocaloric diets higher in protein and lower in carbohydrate are beneficial in restoring insulin sensitivity and promoting fat loss in cats (Rand et al. 2004; Hoenig et al. 2007). Insulin resistance and obesity are linked to development of non-insulin-dependent (type-II) diabetes mellitus, and cats are considerably more prone than dogs to development of type-II diabetes (Hoenig 2002; Rand et al. 2004).

High rates of gluconeogenesis in cats require a steady supply of precursors. Gluconeogenesis includes extensive recycling of glucose via lactate and alanine, as well as de novo production of glucose from non-carbohydrate precursors (Katz and Tayek 1998, 1999). In cats, the only quantitatively significant precursors available for de novo glucose synthesis are amino acids, followed by glycerol. Glycerol is both readily converted to glucose and relatively abundant as a constituent of dietary and endogenous triglyceride (Streja et al. 1977), but its utilisation as a gluconeogenic substrate is limited by the need to oxidise the associated free fatty acids. Therefore, the principal substrates for glucose synthesis available to cats and other hypercarnivores are amino acids.

Amino acid metabolism

The principal metabolic fates of amino acids include protein synthesis, synthesis of other essential compounds (e.g. creatine, purine bases), and oxidative catabolism to glucose, ketone bodies, and carbon dioxide (Jungas et al. 1992; Waterlow 2006). Nitrogen generated during amino acid catabolism is converted to urea and excreted; other minor nitrogenous end products of mammals include ammonia and uric acid (Wright 1995). In omnivores, amino acid oxidation accounts for a small proportion of total energy expenditure, and assuming that intake of energy is adequate, use of amino acids for maintenance of

the body protein mass takes priority over the use of amino acids as fuel (Moundras et al. 1993; Millward 1995).

Degradation of amino acids involves two metabolic cycles, the urea cycle and the tricarboxylic acid (TCA) cycle. While the TCA cycle is common to all cells with mitochondria, the urea cycle occurs almost exclusively in the liver (Morris 2002), which is also the principal site of gluconeogenesis. The urea cycle is functionally linked to gluconeogenesis via TCA cycle intermediates, resulting in the coordination of urea formation and gluconeogenesis (Jungas et al. 1992). After deamination, the nitrogen moiety of amino acids enters the urea cycle, while residual carbon skeletons are converted into acetyl-CoA or acetoacetyl-CoA, pyruvate, and TCA cycle intermediates (Table 3). Acetyl-CoA and acetoacetyl-CoA cannot be converted to glucose, but may be oxidised directly in the TCA cycle, used for ketogenesis, or for the synthesis of fatty acids or cholesterol. Net lipogenesis from amino acids is considered a minor pathway in general (Jungas et al. 1992; Welle 1999). The branched-chain amino acids (leucine, isoleucine, valine) are absorbed primarily by muscle, where they are catabolised by transamination (usually to glutamate) followed by oxidation. Since oxaloacetate is a key intermediate in gluconeogenesis, all amino acids that are catabolised via pyruvate or TCA cycle intermediates are potentially gluconeogenic; only leucine and lysine are exclusively ketogenic (Table 3).

Table 3 Principal intermediates in the oxidative disposal of protein amino acids according to Berg et al. (2002)

Amino acid	Metabolic intermediate
Alanine	Pyruvate
Arginine	α-Ketoglutarate*
Asparagine	Oxaloacetate*
Aspartic acid	Oxaloacetate*, fumarate*
Cysteine	Pyruvate
Glutamine	α-Ketoglutarate*
Glutamic acid	α-Ketoglutarate*
Glycine	Pyruvate
Histidine	α-Ketoglutarate*
Isoleucine	Acetyl-CoA, succinyl-CoA*
Leucine	Acetyl-CoA, acetoacetyl-CoA
Lysine	Acetoacetyl-CoA
Methionine	Succinyl-CoA*
Phenylalanine	Acetoacetyl-CoA, fumarate*
Proline	α-Ketoglutarate*
Serine	Pyruvate
Threonine	Pyruvate, succinyl-CoA*
Tryptophan	Pyruvate, acetyl-CoA, acetoacetyl-CoA
Tyrosine	Acetoacetyl-CoA, fumarate*
Valine	Succinyl-CoA*

* Tricarboxylic acid cycle intermediate

The majority of amino acids are catabolised via pyruvate or TCA intermediates (Table 3), resulting in entry of 4- and 5-carbon intermediates into the TCA cycle (*anaplerosis*). For every turn of the cycle, one 2-carbon unit enters as acetyl-CoA and two 1-carbon units leave as CO_2. While pyruvate can enter the cycle as acetyl-CoA after decarboxylation by pyruvate dehydrogenase, compounds containing four or five carbons cannot be fully oxidised in the TCA cycle, and the anaplerotic influx of carbon to the cycle has to be balanced by an equivalent removal of carbon (*cataplerosis*) to maintain cycle function (Owen et al. 2002). This is achieved by routing oxaloacetate and other TCA intermediates into metabolic pathways such as gluconeogenesis via phosphoenolpyruvate carboxykinase (Owen et al. 2002; Hakimi et al. 2005). Channelling of amino acid skeletons into gluconeogenesis and ketogenesis not only maintains TCA cycle function (Owen et al. 2002; Hakimi et al. 2005), but also converts a large range of amino acids into *universal metabolic currency* (glucose, ketone bodies) that can be taken up and utilised by other tissues (Jungas et al. 1992), many of which do not possess the enzymatic machinery for metabolising amino acids (Jungas et al. 1992) or which, like the brain, cannot take up amino acids at sufficient rates (Sokoloff et al. 1977). In humans, conversion of amino acids to substrates that can be exported and oxidised by extrahepatic tissues is considered necessary to reduce the functional strain on liver metabolism, as complete postprandial oxidation of dietary amino acids by the liver would produce surplus ATP and exceed hepatic capacity for oxidative metabolism (Jungas et al. 1992). This constraint must apply to an even greater extent to hypercarnivores, given that their protein intakes are much higher than that of most human populations. Based on in vitro studies of cat hepatocytes, Beliveau and Freedland (1982) concluded that amino acids are protected from complete oxidation and preferentially directed into synthetic pathways (gluconeogenesis, protein synthesis) in cats. Glucose is not a precursor for fatty acid synthesis in cat liver cells (Richard et al. 1989), which would increase the availability of glucose as a substrate for extrahepatic tissues. Thus, gluconeogenesis is maximal in carnivores in the postprandial phase (shortly after a meal), whereas in omnivores, gluconeogenesis is maximal once the animal is postabsorptive or fasting (MacDonald et al. 1984). This may also explain why the preferred meal pattern of the cat is frequent, small meals distributed throughout its activity cycle (Bradshaw 2006).

In summary, glucose synthesis is one of the principal means of oxidative amino acid disposal, and consumption of protein is necessarily accompanied by gluconeogenesis from amino acids. However, the question remains whether

gluconeogenesis in the cat is simply a means of disposing of excess amino acids (i.e. amino acids not required for the maintenance of body protein mass or other specific synthetic processes), or whether gluconeogenesis from amino acids continues despite relative protein deficiency and the threat of a negative nitrogen balance. In rats on a high-protein diet (60% casein by mass), the utilisation of dietary amino acids for gluconeogenesis may limit their availability for protein synthesis (Moundras et al. 1993). In humans, pigs, dogs, and rats, fasting amino acid oxidation (FUNL) is substantially higher than the obligatory amino acid oxidation (EUNL) in the fed state (Table 1). The catabolism of additional amino acids provides substrates for gluconeogenesis during total starvation. These examples show that under certain conditions, glucose production takes metabolic priority over conservation of body protein, and lend support to the possibility that the protein requirement of cats is increased above that of omnivorous mammals as a result of homeorhetic partitioning of amino acids into obligatory gluconeogenesis. In keeping with this, Silva and Mercer (1985) suggested that cats are "wasteful" of amino acids at low protein intakes due to the high priority of gluconeogenesis. Furthermore, cats do not have a high requirement for indispensable amino acids, but for total amino nitrogen (Rogers and Morris 1979), consistent with utilisation of amino acids for gluconeogenesis, not net protein synthesis.

Protein turnover and nitrogen loss

Body proteins are constantly 'turned over' that is synthesised and broken down (Welle 1999; Waterlow 2006). A proportion of the amino acids released by the breakdown of body protein is oxidised rather than recycled back into protein synthesis, and nitrogen is continually lost from the body in urine and faeces, as shed skin and hair, and in skin gland secretions. Nitrogen loss scales with protein intake and on a protein-free but otherwise adequate diet, daily nitrogen loss decreases to a minimum value after a period of adaptation (Hoffer 1999). This minimum nitrogen loss is called the obligatory nitrogen loss or ONL (Hoffer 1999). Because nitrogen loss from the body has to be replaced by dietary intake, the ONL sets the lower limit for the amount of protein required to maintain nitrogen balance (Waterlow 1999). In humans, the appropriate nitrogen intake to balance losses is estimated as 130–140% of the ONL (Young et al. 1989).

Due to the practical challenge of collecting every last bit of nitrogen lost from the body, EUNL or total endogenous nitrogen loss (EUNL plus metabolic faecal nitrogen) is commonly measured instead of ONL (Kendall et al. 1982; Flurer et al. 1988; Hendriks et al. 1997; Owen et al. 1998; Green et al. 2008). In the basal state, EUNL accounts for the majority of ONL from the body (Table 1), and represents oxidative catabolism of amino acids as opposed to loss of nitrogen by other means (e.g. as shed hair, skin, or other cellular debris). During the transition to total starvation, amino acids are directed towards gluconeogenesis to meet endogenous glucose demands to compensate for the absence of dietary carbohydrate, nitrogen loss increases accordingly, and thus FUNL generally exceeds ONL and EUNL in mammals (Lusk 1928; Nielsen et al. 1994; Henry and Collingwood 1998; Table 1). In long-term starved humans, the FUNL (240–416 mg N $kg^{-0.75}$ day^{-1}) is two to four times greater than estimated EUNL ($\sim$100 mg N $kg^{-0.75}$ day^{-1}) due to use of amino acids for gluconeogenesis during fasting (Göschke et al. 1975; Henry and Collingwood 1998; Owen et al. 1998; Hoffer 1999; Table 1); feeding small quantities of carbohydrate significantly reduces urinary nitrogen loss by decreasing gluconeogenesis from amino acids (Owen et al. 1998). In contrast, the measured EUNL of cats of 360 mg N $kg^{-0.75}$ day^{-1} (Miller and Allison 1958; Hendriks et al. 1997) is considerably higher than the mean FUNL of cats fasted for 1–6 weeks of 283 mg N $kg^{-0.75}$ day^{-1} (Biourge et al. 1994; Table 1). Because EUNL provides an approximation of amino acid oxidation, this observation provides further support for the proposition that in cats, minimal rates of gluconeogenesis from amino acids in the fed state are as high as, or even higher than, during total starvation (Rogers et al. 1977; Kettelhut et al. 1980). Constitutively high rates of gluconeogenesis make sense if ensuring an uninterrupted supply of glucose is a metabolic priority, e.g. to support an organ system (brain, mammary gland, conceptus) that absolutely requires glucose to function.

The brain: glucose demands in relation to brain size

Brain tissue does not tolerate any interruption of its fuel supply and its default fuel is glucose (Sokoloff et al. 1977; Hertz and Dienel 2002), although partial substitution by ketone bodies may occur during prolonged starvation (Cahill et al. 1966; Owen et al. 1967). Brain glucose demand depends primarily on brain mass, and there are large differences among mammals in the proportion of whole-body glucose demand used by the brain as a result of the allometry of brain size and taxonomic differences in encephalisation. For example, uptake of glucose by the brain accounts for 40–50% of whole-body glucose turnover in humans, whose brain represents 2% of body mass, but for only ca. 10% of whole-body glucose turnover in the sheep with a brain that accounts for only 0.2% of body mass (Pell and Bergman 1983; Ekberg et al. 1999). In humans, changes in brain glucose demand from birth to

Table 4 Comparison of relative brain size, brain metabolic rate (CMR_{glu}), and estimated brain glucose demand in cats and other mammals

Species	Body mass (kg)	Brain mass (g)	Brain (% of BM)	CMR_{glu} (μmol 100 g^{-1} min^{-1})	Brain glucose demand	
					Absolute (g day^{-1})*	Relative (RCMR) (g kg $BM^{-0.75}$ day^{-1})
Published values (Fig. 1)						
Mouse (*Mus musculus* dom.)	0.0183[a]	0.446[a]	2.44	72[b,c]	0.083	1.7
Ground squirrel (*Spermophilus tridecemlineatus*)	0.146[d]	2.39[d,e]	1.64	60[f]	0.37	1.6
Rat (Wistar) (*Rattus norvegicus* dom.)	0.205[g]	1.75[h]	0.85	65[i]	0.29	0.96
Cat (*Felis silvestris* dom.)	3.41[j]	30.2[j]	0.89	40[k]	3.1	1.2
Dog (*Canis lupus* dom.)	19.9[l]	85.2[l]	0.43	34[m]	7.5	0.80
Sheep (*Ovis aries* dom.)	57.5[n]	114[n]	0.20	35[n]	10	0.50
Human (*Homo sapiens*)	70.0[o]	1,400[o]	2.0	26[o] (30[o,p])[†]	94 (110)[†]	3.9 (4.5)[†]
Weddell seal (*Leptonychotes weddellii*)	376[q]	563[q]	0.15	28[r]	41	0.48
Predicted values A: Cat-sized eutherians from different orders						
Rabbit (*Oryctolagus cuniculus* dom.)	3.68[s]	10.6[s]	0.29	48	1.3	0.49
Armadillo (*Dasypus novemcinctus*)	3.40[s]	7.5[s]	0.22	50	0.97	0.39
Woodchuck (*Marmota monax*)	4.20[t]	11.2[t]	0.27	47	1.4	0.47
Rock hyrax (*Procavia capensis*)	3.80[u]	20.5[u]	0.54	44	2.3	0.85
Dik-dik (*Madoqua kirki*)	4.60[v]	37[v]	0.80	40	3.9	1.2
Capuchin monkey (*Cebus capuchin*)	3.10[s]	72.2[s]	2.3	38	7.1	3.0
Predicted values B: Carnivora from 0.2 to 365 kg						
Stoat (*Mustela erminea*)	0.185[w]	4.5[w]	2.4	54	0.62	2.2
Mink (*Mustela vison* dom.)	1.56[x]	9.35[x]	0.62	49	1.2	0.84
Mountain lion (*Puma concolor*)	50.4[y]	125[y]	0.25	34	11	0.59
Tiger (*Panthera tigris*)	159[y]	279[y]	0.18	31	22	0.50
Polar bear (*Ursus maritimus*)	365[y]	459[y]	0.13	29	34	0.41

Published values were limited to whole-brain CMR_{glu} of adults. Predicted values of CMR_{glu} were calculated using the allometric relationship shown in Fig. 1. Domestic species are indicated by 'dom.'

* Calculated from brain CMR_{glu} and brain mass, [†] values for net and (total) CMR_{glu}, see text for explanation

[a] Khan et al. (1999), [b] Growdon et al. (1971), [c] Kamp et al. (1980), [d] Iwaniuk (2001), [e] brain mass calculated from brain volume in ref. d according to Palombo et al. (2008), [f] Frerichs et al. (1995), [g] Hawkins et al. (1974), [h] Even et al. (2001), [i] mean of values from Hawkins et al. (1974) and Nehlig and Pereira de Vasconcelos (1993), [j] Röhrs and Ebinger (1999), [k] Harper and Jennett (1990), [l] Bronson (1979), [m] mean of values reported in Gregoire et al. (1978), [n] Pell and Bergman (1983), [o] Sokoloff et al. (1977), [p] Kemppainen et al. (2005), [q] Bininda-Emonds and Gittleman (2000), [r] Murphy et al. (1980), [s] Crile and Quiring (1940), [t] Meier (1983), [u] Sacher and Staffeldt (1974), [v] Pérez-Barbería and Gordon (2005), [w] Kruska (2005), [x] Kruska (1993), [y] Gittleman (1986)

adulthood are thought to be the principal determinant of hepatic glucose production (Bier et al. 1977).

Within a mammalian order, relative brain mass as a percentage of body mass generally decreases with increasing species size (Eisenberg 1981; Kruska 2005). Mammalian orders differ in their degree of encephalisation so that the brain mass predicted at a given body mass is greater in the Carnivora and Artiodactyla than in Insectivora and Rodentia (Kruska 2005). For example, the stoat (*Mustela erminea*) weighs approximately the same as a Norway rat (*Rattus norvegicus*) but has a brain that has twice the mass of rat brain (Kruska 2005; Table 4). Lastly, not only does brain mass vary with body mass and taxonomic order, but the metabolic rate of the brain also decreases allometrically with brain mass (Mink et al. 1981; Karbowski 2007), rather than being constant as previously assumed (Grande 1980; Kuzawa 1998). The relationship between cerebral glucose utilisation, CMR_{glu}, and total brain mass is shown in Fig. 1 and Table 4. Similar relationships have been found for brain metabolic rate versus brain volume (Karbowski 2007) and for oxygen consumption versus mass of the vertebrate central nervous system (Mink et al. 1981). Thus, within a taxon of comparable degree of encephalisation, smaller species have not only relatively larger brains, but also brains with a higher mass-specific metabolic rate; at the same time, whole-body metabolic rate (and therefore the capacity to supply substrates to the brain) increases with decreasing species size.

To compare the glucose demand of the cat brain with that in other mammalian species, a predictive allometric model was derived from published data of brain mass and whole-brain glucose utilisation (CMR_{glu}) for adult mammals ranging from mouse to Weddell seal (Table 4; Fig. 1; $n = 8$ species). In humans, non-lethal techniques such as arteriovenous difference and positron emission tomography are used, and these methods tend to overestimate glucose uptake relative to the terminal 2-deoxyglucose method used in animal studies (Sokoloff et al. 1977). A value of 29–31 μmol glucose 100 g^{-1} min^{-1} is commonly assumed for human brain, but net uptake of glucose in human brain is closer to 26 μmol 100 g^{-1} min^{-1} (Sokoloff et al. 1977) and therefore this lower CMR_{glu} value for human brain was used to allow comparison with animal data (Table 4). The accuracy of prediction from the resulting allometric model was tested by computing the regression for $n - 1$ values, using the regression parameters to predict the nth value, and repeating this process to calculate an independent predicted value for each species that was then compared to the actual published value (Dielman 2005). The median difference between predicted and published glucose utilisation values was −1% of the actual value (range −7 to +8%), and actual and predicted values were not significantly different (paired t-test, $P = 0.9$). A Deming linear regression model (Linnet 1998) incorporating all published data (Fig. 1) was then used to predict brain glucose requirements for (a) cat-sized species from a range of mammalian orders (Rodentia, Lagomorpha, Xenarthra, Hyracoidea, Artiodactyla, Primates), and (b) hypercarnivorous members of the order Carnivora with body masses ranging from 0.2 to 365 kg (stoat, mink, mountain lion, tiger, and polar bear; Table 4). Estimates of brain glucose requirements were expressed as absolute demand (mass of glucose per day) and as relative brain glucose demand (RCMR) expressed on the basis of metabolic body mass ($BM^{0.75}$). RCMR is an index of brain glucose demand relative to the organism's metabolic capacity to supply this demand.

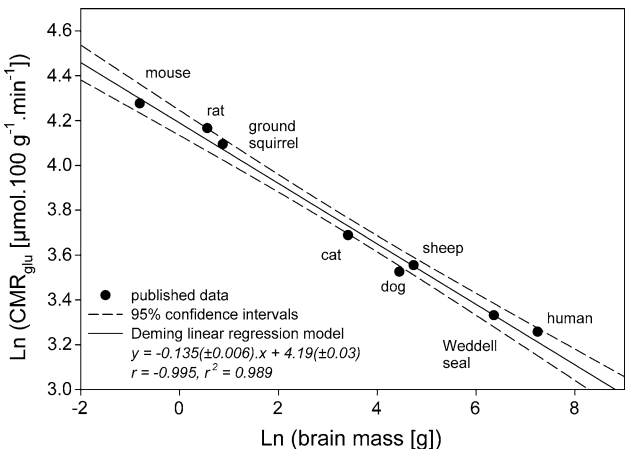

Fig. 1 Allometric scaling of cerebral metabolic rate (glucose utilisation, CMR_{glu}) with brain mass in adult conscious mammals. Data and sources, see Table 4

The results of this analysis are shown in Table 4. Firstly, RCMR of cat brain is only exceeded by a primate among mammals in the same size range, consistent with the fact that members of the order Carnivora are highly encephalised. Estimated relative brain glucose demand expressed on a metabolic-mass basis (RCMR), as proposed here, correlates well with known differences in encephalisation between mammalian orders (Kruska 2005) and may be a more useful metric than the encephalisation quotient for comparative purposes; it not only takes into account the allometric scaling of brain metabolism, but also avoids the problems associated with scaling of brain mass across mammalian taxa that affect the concept of the encephalisation quotient (Gittleman 1986; Pagel and Harvey 1989; Kruska 2005).

Secondly, cats have a high brain glucose requirement compared with larger species of Carnivora (Table 4). In keeping with this greater need of cats for brain substrates, the cat develops hyperketonaemia more rapidly and to a greater degree than the dog during total starvation (Owen et al. 1967; De Bruijne 1979; Blanchard et al. 2002). A special case is the mink (*M. vison*) in that predicted RCMR of adult

mink is similar to that of a cat, even though the mink is a considerably smaller species and would be predicted to have a relatively larger brain. The explanation for this anomaly is that mink undergo an absolute reduction in brain mass and volume during the later stages of ontogeny (Kruska 1993), and thus adults have a smaller brain and lower brain glucose requirement than may be expected based on body mass (Table 4). Currently, there is no explanation for this phenomenon, although a reduction in brain mass may convey an adaptive advantage for small, semi-aquatic hypercarnivores by reducing brain glucose demands.

As a result of having a relatively large brain, cats indeed have a large endogenous glucose demand (Table 4) that needs to be met through gluconeogenesis on a hypercarnivorous diet. The net brain glucose demand of cats listed in Table 4 represents ca. 30% of measured gluconeogenesis of cats after an overnight fast (Kley et al. 2009). This is a large proportion considering that gluconeogenesis includes significant recycling of glucose and that the equivalent value for humans, with their much larger brain, is ca. 44% (Ackermans et al. 2001). The question remaining is whether the estimated glucose requirement of the cat brain is sufficient to explain the elevated protein requirements of cats.

Contribution of gluconeogenesis to protein requirements

As a result of the functional interdependency of urea production and gluconeogenesis from amino acids, urinary nitrogen loss provides an estimate of hepatic amino acid oxidation (Welle 1999), and hence an upper limit for the rate of gluconeogenesis from amino acids. The EUNL of rats and humans represents a minimal estimate for oxidation of amino acids released from the breakdown of body proteins due to turnover. The hypothesis presented in this paper is that in cats and other hypercarnivores, the lower threshold value for amino acid oxidation is determined by the endogenous glucose demand of the brain and other tissues (obligatory gluconeogenesis). Because cats have relatively large brains, obligatory gluconeogenesis from amino acids should result in endogenous nitrogen losses that are higher than predicted for a cat-sized, non-hypercarnivorous mammal. This proposition can be tested by comparing observed EUNL values (as a proxy for total amino acid oxidation) with the theoretical nitrogen cost of meeting the endogenous glucose demand entirely from amino acid-based gluconeogenesis. According to the proposed model, the estimated minimum nitrogen cost of gluconeogenesis should match observed EUNL in hypercarnivores cats and mink, but not in humans and rats, since in the latter endogenous nitrogen loss is limited to losses incurred due to protein turnover, and metabolic glucose demands in the fed state are primarily met through dietary carbohydrate.

In the absence of quantitative data on whole-body glucose demand of cats and mink, brain glucose data (Table 4) were used to derive a minimal estimate of the endogenous glucose demand and the associated nitrogen cost. As shown in Table 5, there is close agreement between nitrogen loss

Table 5 Comparison of endogenous urinary nitrogen loss (EUNL) with brain glucose demand in (a) hypercarnivores cat and mink and in (b) omnivores rat and human

Parameter	Cat	Mink	Rat	Human
Theoretical nitrogen costs of brain glucose demand				
Body mass (kg)	3.41[a]	1.10[b]	0.205	70.0[d]
Metabolic body mass ($kg^{0.75}$)	2.51	1.07	0.305	24.2
Brain mass (g)	30.2[a]	7.70[c]	1.75	1,400[d]
Brain glucose demand (g day^{-1})	3.1[e]	1.0[e]	0.29[e]	94[e]
Protein equivalent (amino acids to glucose) (g day^{-1})	5.5[f]	1.7[f]	0.51[f]	165[f]
Theoretical nitrogen cost of glucose (mg N $kg^{-0.75}$ day^{-1})	350	260	270	1,094
Comparison with reported nitrogen loss				
EUNL (mg N $kg^{-0.75}$ day^{-1})	360[g,h]	280[b]	122[i,j]	105[k,l]
EUNL (mg N day^{-1})	902	301	37.2	2,542
Protein equivalent of EUNL (g day^{-1})	5.6	1.9	0.23	15.9
Glucose equivalent of EUNL (g day^{-1})	3.2	1.1	0.13	9.1
Theoretical nitrogen cost of brain glucose demand relative to reported endogenous urinary nitrogen loss (%)	97	93	221	1042

Protein is assumed to contain 16% nitrogen

[a] Röhrs and Ebinger (1999), [b] Tauson et al. (2001), [c] Kruska 1993, [d] Sokoloff et al. 1977, [e] Table 4, this paper, [f] Jungas et al. (1992), [g] Hendriks et al. (1997), [h] Miller and Allison (1958), [i] Uezu et al. (1983), [j] Hendriks et al. (1997), [k] Calloway and Margen (1971), [l] Scrimshaw et al. (1972)

predicted from brain glucose demand and reported EUNL for both cats and mink. By contrast, the theoretical protein cost of brain glucose requirements greatly exceeds observed EUNL values in rats and humans, because in the fed state endogenous nitrogen loss is determined by protein turnover, not by glucose demand.

The relationship between brain glucose demand and EUNL shown in Table 5 strongly supports the hypothesis that high endogenous nitrogen losses in cats and mink are the consequence of augmentation of the ONL sensu stricto by constitutive protein-based glucose production, or *obligatory gluconeogenesis*. The brain is not the only tissue with an absolute glucose requirement, but cats have large brains that are likely to account for at least half of the obligatory glucose demand in the non-reproductive, resting state. Underestimation of the whole-body glucose demand of cats by considering only the needs of the brain is offset to some degree by the fact that cats will derive a proportion of their endogenous glucose production from glycerol, as discussed above. Pending further studies, the model of endogenous nitrogen loss in cats presented here must be considered hypothetical. But if the protein requirements of cats and other small hypercarnivores are indeed determined by two factors, obligatory gluconeogenesis and protein turnover, then this explains not only the high protein requirement of cats and the observed discrepancy between rates of protein synthesis and protein oxidation (Russell et al. 2003), but also the question of why fasting nitrogen excretion in cats is lower than the EUNL (Table 1). The discrepancy resolves if it is assumed that all or most of the cat's endogenous glucose demand is synthesised from amino acids in the fed state. During the transition to prolonged starvation, cats develop increasing hyperketonaemia (Blanchard et al. 2002). Partial replacement of brain glucose by ketone bodies (Owen et al. 1967) permits reduction of the rate of gluconeogenesis from protein, and thus results in a decrease of nitrogen excretion.

Considering possible objections to the model proposed here, two obvious points are: (1) obligatory gluconeogenesis carries a metabolic cost; and (2) why should obligatory gluconeogenesis be maintained in domestic cats provided with high-carbohydrate, low-protein foods by humans?

1. While obligatory gluconeogenesis releases hypercarnivores from any dependence on dietary carbohydrate, a potential disadvantage is that it reduces metabolic flexibility and renders the animal incapable of adapting to diets low in protein. However, cats evolved to consume a highly digestible diet containing 30% or more of ME as protein and less than 5% of ME as carbohydrate (Table 2). On such a diet, the risks associated with a transiently negative nitrogen balance as a result of obligatory gluconeogenesis are small relative to the acute threat of impaired function of the brain and other organ systems due to hypoglycaemia. It follows that for a hypercarnivore, not only there is no adaptive advantage in developing a tolerance for low-protein diets, but also there is a need to maintain the capacity for high rates of protein-based gluconeogenesis.
2. Even if wild felids rarely consume carbohydrate in nature, the domestic cat consumes a large proportion of human-supplied foods containing 15–35% of ME as carbohydrate (Table 2). Thus, domestication would seem to favour cats that can adapt to carbohydrate loads by reducing obligatory gluconeogenesis from protein; on the other hand, it is not clear that the selection pressures cats have been subject to have been sufficient to force this kind of metabolic reassessment. Systematic feeding of cats, although ritually practiced by the ancient Egyptians (MacDonald et al. 1984), is a phenomenon of the twentieth century (Siewert 2003). For most of their history together, humans have contributed little to the feeding of cats (Siewert 2003; Driscoll et al. 2009), and to this day cats will supplement their diet with prey (Liberg 1984; Weber and Dailly 1998; Baker et al. 2005; Biró et al. 2005), unless confined indoors in rodent-free premises (e.g. research laboratories). Thus, *the diet to which the modern domestic cat is habituated* is a mixture of human-supplied food and high-protein vertebrate prey, and is likely to contain a least 30% of ME as protein (Table 2). Farmed mink, another domesticated hypercarnivore, are fed diets that contain 30–40% of ME as protein to promote fur production (Damgaard et al. 1998). It follows that failure to adapt to provision of dietary carbohydrate by reducing obligatory gluconeogenesis from protein is unlikely to carry the risk of protein deficiency in cats and mink, and continued exposure to large dietary protein loads provides an ongoing incentive to maintain the metabolic status quo. However, as discussed above, there is evidence to suggest that excess dietary carbohydrate increases the risk of obesity and diabetes in pet cats.

One consequence of the composite ONL of hypercarnivores proposed here is that it alters the relationship between metabolic mass and endogenous nitrogen loss. It has been known for a long time that endogenous nitrogen loss in mammals (ONL and EUNL) is proportional to basal metabolic rate and metabolic body mass, $BM^{0.75}$ (Smith 1926; Lusk 1928; Brody 1945; Kleiber 1975; Henry and Collingwood 1998). But if endogenous nitrogen loss in hypercarnivores is in fact an incremental combination of two factors, one (the nitrogen cost of protein turnover) expected to scale with metabolic body mass and the other

(the nitrogen cost of glucose synthesis) expected to scale with brain mass, then the ONL of hypercarnivores is predicted to increase with decreasing species size, because brain mass and brain metabolic rate do not scale with metabolic mass (Eisenberg 1981; Gittleman 1986; Kruska 2005; Fig. 1; Table 4). Conversely, in very large hypercarnivores, the basal rate of oxidation of amino acids due to protein turnover is likely to be sufficient to meet endogenous glucose demands, making catabolism of additional amino acids for gluconeogenesis unnecessary.

Summary and outlook

Cats have evolved a high capacity for de novo production of glucose from amino acids to solve the problem of how to thrive on a hypercarnivorous diet while being a small mammal with a large brain. According to the model presented here, cats do not have a high protein requirement per se, but rather a secondarily elevated protein requirement in response to a high endogenous glucose demand, thus resolving the paradox identified by Russell et al. (2003).

Constitutively high rates of gluconeogenesis in cats are adaptive on their natural diet, i.e. when little or no carbohydrate is consumed, but may predispose cats to developing hyperglycaemia and insulin resistance, and their metabolic sequelae, on diets that contain significant proportion of ME as carbohydrate. Actual avoidance of excess carbohydrate intake may help explain poor acceptance of synthetic diets offered to cats in anthropogenic settings (Green et al. 2008), and it would be interesting to test whether hypercarnivores balance carbohydrate intake, as has been found for fat and protein (Mayntz et al. 2009). Further study is required to specifically quantify rates of gluconeogenesis, protein synthesis, and protein oxidation in cats fed different levels of protein and carbohydrate. The available evidence suggests that cats do not significantly downregulate gluconeogenesis in response to carbohydrate intake, but this assumption remains without direct experimental support. For example, if gluconeogenesis in cats is suppressed to a small degree by dietary carbohydrate, then this effect may, at marginal intakes of protein, make the difference between positive and negative nitrogen balance. The potential effects of high or variable dietary carbohydrate content in experimental diets need to be considered in the design and interpretation of nutritional and metabolic studies. Given the proposed relationship between endogenous glucose demand and nitrogen metabolism, it would also be interesting to investigate changes in rates of gluconeogenesis and protein requirements in physiological states characterised by an increased glucose demand, such as immaturity, pregnancy, lactation, and trauma.

In summary, the allometric relationship between brain size, metabolic rate, and nitrogen requirements is predicted to follow a different trajectory in hypercarnivores compared with other mammals as a result of the additional nitrogen cost of obligatory gluconeogenesis, a hypothesis that can be tested by comparing hypercarnivores of a similar degree of encephalisation but different body size (e.g. stoat, tiger). Thus, it may be predicted that hypercarnivory in mammals imposes constraints on minimal body size and degree of encephalisation that differ from the effects of metabolic rate alone. It also suggests that care should be taken when using protein requirements of the domestic cat as the basis for predicting requirements of larger hypercarnivores in captivity.

Acknowledgments Much of the work on an earlier draft of this paper was done while I was a fellow at the Smithsonian National Zoological Park, and I thank J. Seidensticker for his encouragement and support of this endeavour. I also wish to thank S. Lumpkin and M. Power for commenting on an earlier version of the manuscript, and J. Noordhof and in particular O. Oftedal for critical discussions of the subject matter. I also thank my anonymous reviewers, D. Millward and especially G. Lobley for their time and constructive comments. This work was supported by a grant from the National Science Foundation-Office of Polar Programs No. 0538592.

Appendix: Total carbohydrate in a mouse

The calculation presented here was intentionally done as a 'best-case scenario' to estimate the maximal amount of digestible carbohydrate a cat could derive from consuming gut contents of small, granivorous rodent prey. Data used in the calculation include body composition of Swiss albino mice (Bailey et al. 1960) and mouse gut volumes from Cizek (1954). Mice were chosen because they are granivorous rather than herbivorous, have a relatively large gut volume (Cizek 1954), and body composition data are readily available. It is assumed that gut contents consist entirely of finely ground whole wheat grain (composition of dry matter: 11.2% fibre, 73.9% starch and dextrins, 2.6% sugars: Paul and Southgate 1978), and that digestibility of starch in gut contents is 97.2% in cats (Morris et al. 1977). It is further assumed that cats cannot digest non-starch polysaccharides to a significant extent (NRC 2006) but that simple sugars are completely digested. BM, body mass.

Glycogen in liver and muscle

(a) Liver mass and glycogen content

Liver mass of adult mice = ca. 5% of body mass (Von Ehrenstein 1958; Barnett and Widdowson 1965; Chaffee et al. 1966).
Liver glycogen content in mice (Ryder et al. 1999): 100–400 μmol glucose g^{-1} liver wet mass, equivalent to 1.8–7.2 g glucose 100 g^{-1} liver.

(b) Skeletal muscle mass and glycogen content

Skeletal muscle mass in rats is ca. 64% of lean body mass and ca. 45% of BM (Even et al. 2001); this is consistent with ash-free, fat-free carcass mass (an approximation of total skeletal muscle mass) in mice of ca. 44% of BM (Corva and Medrano 2000).

Muscle glycogen content (Ryder et al. 1999): 20 µmol glucose g^{-1} wet muscle, 0.4 g glucose 100 g^{-1} wet muscle.

Please note that mouse muscle contains considerably less glycogen than human muscle tissue (0.2–0.4 vs. 2%).

Combining (a) and (b):

Assume mouse 22 g, 14% body fat, 18.92 g lean mass

Approximate liver mass (5% BM)	1.1 g
Glycogen in liver (7%)	77 mg
Muscle mass (45% of BM)	9.9 g
Glycogen in muscle (0.4%)	40 mg
Total glycogen	0.124 g

Digestible carbohydrate in the digestive tract of prey

Body mass mouse	22 g
Gut contents (DM)	0.30 g
73.8% starch + dextrins	0.219 g
×0.972	0.212 g
Sugars, 2.6%	0.008 g
Total available CHO	0.220 g
	3.69 kJ

Summary: total carbohydrate (CHO)

Glycogen in liver and muscle (see also Table 2)	0.124 g
Available carbohydrate in gut contents (above)	0.220 g
Total available CHO (×16.76 kJ g^{-1})	0.344 g 5.76 kJ
Total ME of mouse (see Table 2)	8.60 kJ g^{-1} 188.3 kJ
Carbohydrate as %ME	(5.76/188.3) × 100 = 3.1%
In 6 mice (equivalent to daily ME)	
Total ME	1,135.2 kJ
ME from carbohydrate	34.6 kJ
Total carbohydrate	2.1 g

Even in the best-case scenario presented here, consumption of gut contents would contribute insufficient glucose equivalents to meet estimated whole-body carbohydrate demand (the brain alone consumes ca. 3 g glucose day^{-1}; Table 4), assuming that cats actually consume gut contents. Prey larger than mice, such as rabbits, tend to be herbivorous rather than granivorous and possess fermentative digestion; it is questionable whether cats would consume partially digested herbage and if they did, it is unlikely cats could derive significant carbohydrate from this source. In general, neither cats (Leyhausen 1979) nor large felids such as tigers (*Panthera tigris*) and mountain lions (*Puma concolor*) consume the gut contents of their prey (J. Seidensticker, pers. comm., 01 June 2009).

References

Ackermans MT, Pereira Arias AM, Bisschop PH, Endert E, Sauerwein HP, Romijn JA (2001) The quantification of gluconeogenesis in healthy men by $^{2}H_2O$ and [2-^{13}C]glycerol yields different results: rates of gluconeogenesis in healthy men measured with $^{2}H_2O$ are higher than those measured with [2-^{13}C]glycerol. J Clin Endocrinol Metab 86:2220–2226

Appleton DJ, Rand JS, Sunvold GD (2001) Insulin sensitivity decreases with obesity, and lean cats with low insulin sensitivity are at greatest risk of glucose intolerance with weight gain. J Feline Med Surg 3:211–228

Astrup A, Larsen TM, Harper A (2004) Atkins and other low-carbohydrate diets: hoax or an effective tool for weight loss? Lancet 364:897–899

Ayala MDR, Racotta R, Hernandez-Montes H, Quevedo L (2006) Some metabolic effects on lactating rats of a low-energy diet restricted in good-quality protein. Br J Nutr 96:667–673

Bailey CB, Kitts WO, Wood AJ (1960) Changes in the gross chemical composition of the mouse during growth in relation to the assessment of physiological age. Can J Anim Sci 40:143–155

Baker PJ, Bentley AJ, Ansell RJ, Harris S (2005) Impact of predation by domestic cats *Felis catus* in an urban area. Mammal Rev 35:302–312

Barnett SA, Widdowson EM (1965) Organ weights and body composition in mice bred for many generations at −3°C. Proc R Soc Lond B 162:502–516

Beliveau GP, Freedland RA (1982) Metabolism of serine, glycine and threonine in isolated cat hepatocytes *Felis domestica*. Comp Biochem Physiol B 71:13–18

Belo PS, Romsos DR, Leveille GA (1976) Influence of diet on glucose tolerance, on the rate of glucose utilization and on gluconeogenic enzyme activities in the dog. J Nutr 106:1465–1474

Berg JM, Tymoczko JL, Stryer L (2002) Biochemistry, 5th edn. W. H. Freeman and Co., New York

Bier DM, Leake RD, Haymond MW, Arnold KJ, Gruenke LD, Sperling MA, Kipnis DM (1977) Measurement of "true" glucose production rates in infancy and childhood with 6,6-dideutero-glucose. Diabetes 26:1016–1023

Bininda-Emonds ORP, Gittleman JL (2000) Are pinnipeds functionally different from fissiped carnivores? The importance of phylogenetic comparative analyses. Evolution 54:1011–1123

Biourge V, Groff JM, Fisher C, Bee D, Morris JG, Rogers QR (1994) Nitrogen balance, plasma free amino acid concentrations and urinary orotic acid excretion during long-term fasting in cats. J Nutr 124:1094–1103

Biró Z, Lanszki J, Szemethy L, Heltai M, Randi E (2005) Feeding habits of feral domestic cats (*Felis catus*), wild cats (*Felis silvestris*) and their hybrids: trophic niche overlap among cat groups in Hungary. J Zool 266:187–196

Blanchard G, Paragon BM, Milliat F, Lutton C (2002) Dietary L-carnitine supplementation in obese cats alters carnitine metabolism and decreases ketosis during fasting and induced hepatic lipidosis. J Nutr 132:204–210

Blaxter KL (1962) The fasting metabolism of adult wether sheep. Br J Nutr 16:615–626

Blem CR (1976) Patterns of lipid storage and utilization in birds. Am Zool 16:671–684

Blomqvist G, Alvarsson M, Grill V, von Heijne G, Ingvar M, Thorell J-O, Stone-Elander S, Widen L, Ekberg K (2002) Effect of acute hyperketonemia on the cerebral uptake of ketone bodies in non-diabetic subjects and IDDM patients. Am J Physiol 283:E20–E28

Bradshaw JWS (2006) The evolutionary basis for the feeding behavior of domestic dogs (*Canis familiaris*) and cats (*Felis catus*). J Nutr 136:1927S–1931S

Brody S (1945) [reprinted 1974] Bioenergetics and growth, with special reference to the efficiency complex in domestic animals. Reinhold Publishing Corp, New York [reprint publisher Hafner Press, New York]

Bronson RT (1979) Brain weight-body weight scaling in breeds of dogs and cats. Brain Behav Evol 16:227–236

Cahill GF Jr (1983) Starvation. Trans Am Clin Climatol Assoc 94:1–21

Cahill GJ, Herrera M, Morgan A, Soeldner J, Steinke J, Levy P, Reichard GJ, Kipnis D (1966) Hormone–fuel interrelationships during fasting. J Clin Invest 45:1751–1769

Calloway DH, Margen S (1971) Variation in endogenous nitrogen excretion and dietary nitrogen utilization as determinants of human protein requirement. J Nutr 101:205–216

Chaffee RR, Hensley JC, Spalding JF (1966) Heritable radiation effects on mouse body and organ weights, fat deposition, cellular enzymes, and blood. Genetics 53:875–882

Chugani HT, Hovda DA, Villablanca JR, Phelps ME, Xu WF (1991) Metabolic maturation of the brain: a study of local cerebral glucose utilization in the developing cat. J Cereb Blood Flow Metab 11:35–47

Cizek LJ (1954) Total water content of laboratory animals with special reference to volume of fluid within the lumen of the gastrointestinal tract. Am J Physiol 179:104–110

Corva PM, Medrano JF (2000) Diet effects on weight gain and body composition in high growth (hg/hg) mice. Physiol Genomics 3:17–23

Crile G, Quiring DP (1940) A record of the body weight and certain organ and gland weights of 3690 animals. Ohio J Sci 40:219–260

Dalrymple EF (2004) Pregnancy toxemia in a ferret. Can Vet J 45:150–152

Damgaard BM, Clausen TN, Dietz HH (1998) Effect of dietary protein levels on growth performance, mortality rate and clinical blood parameters in mink (*Mustela vison*). Acta Agric Scand A Anim 48:38–48

De Bruijne JJ (1979) Biochemical observations during total starvation in dogs. Int J Obesity 3:239–247

Dielman TE (2005) Applied regression analysis: a second course in business and economic statistics. Brooks/Cole Thomson Learning, Belmont, CA

Dierenfeld ES, Alcorn HL, Jacobsen KL (2001) Nutrient composition of whole vertebrate prey (excluding fish) fed in zoos. AZA Nutrition Advisory Group fact sheet: a review. American Zoo and Aquarium Association, Silver Spring, MD

Dobenecker B, Kienzle E, Sallmann H-P, Fuhrmann H (1998) Effect of diet on plasma triglycerides, cholesterol, β-hydroxybutyrate and free fatty acids in cats. J Nutr 128:2648S–2650S

Doreau M, Martin-Rosset W, Barlet J-P (1981) Variations au cours de la journée des teneurs encertains constituants plasmatiques chez la jument pouliniére. Reprod Nutr Dev 21:1–17

Driscoll CA, Macdonald DW, O'Brien SJ (2009) From wild animals to domestic pets, an evolutionary view of domestication. Proc Natl Acad Sci USA 106:9971–9978

Edwards MR, McMurtry JP, Vasilatos-Younken R (1999) Relative insensitivity of avian skeletal muscle glycogen to nutritive status. Domest Anim Endocrinol 16:239–247

Eisenberg JF (1981) The relative size of the mammalian brain. In: The mammalian radiations. An analysis of trends in evolution, adaptation, and behavior. The University of Chicago Press, Chicago, pp 275–283

Ekberg K, Landau BR, Wajngot A, Chandramouli V, Efendic S, Brunengraber H, Wahren J (1999) Contributions by kidney and liver to glucose production in the postabsorptive state and after 60 h of fasting. Diabetes 48:292–298

Even PC, Rolland V, Roseau S, Bouthegourd JC, Tome D (2001) Prediction of basal metabolism from organ size in the rat: relationship to strain, feeding, age, and obesity. Am J Physiol 280:R1887–R1896

Felig P (1973) Maternal and fetal fuel homeostasis in human pregnancy. Am J Clin Nutr 26:998–1005

Féry F, d'Attellis NP, Balasse EO (1990) Mechanisms of starvation diabetes: a study with double tracer and indirect calorimetry. Am J Physiol 259:E770–E777

Flatt JP (1995) Use and storage of carbohydrate and fat. Am J Clin Nutr 61:952S–959S

Flurer CI, Krommer G, Zucker H (1988) Endogenous N-excretion and minimal protein requirement for maintenance of the common marmoset (*Callithrix jacchus*). Lab Anim Sci 38:183–186

Frerichs KU, Dienel GA, Cruz NF, Sokoloff L, Hallenbeck JM (1995) Rates of glucose utilization in brain of active and hibernating ground squirrels. Am J Physiol 268:R445–R453

Gannon MC, Nuttall FQ (2004) Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. Diabetes 53:2375–2382

Gittleman JL (1986) Carnivore brain size, behavioral ecology, and phylogeny. J Mammal 67:23–36

Goodman MN, Lowell B, Belur E, Ruderman NB (1984) Sites of protein conservation and loss during starvation: influence of adiposity. Am J Physiol 246:E383–E390

Göschke H, Stahl M, Tholen H (1975) Nitrogen loss in normal and obese subjects during total fast. Klin Wochenschr 53:605–610

Grande F (1980) Energy expenditures of organs and tissues. In: Kinney J (ed) Assessment of energy metabolism in health and disease. Ross Laboratories, Columbus, pp 88–92

Greaves JP, Scott PP (1960) Nutrition of the cat. Br J Nutr 14:361–369

Green AS, Ramsey JJ, Villaverde C, Asami DK, Wei A, Fascetti AJ (2008) Cats are able to adapt protein oxidation to protein intake provided their requirement for dietary protein is met. J Nutr 138:1053–1060

Gregoire NM, Gjedde A, Plum F, Duffy TE (1978) Cerebral blood flow and cerebral metabolic rates for oxygen, glucose, and ketone bodies in newborn dogs. J Neurochem 30:63–69

Growdon WA, Bratton TS, Houston MC, Tarpley HL, Regen DM (1971) Brain glucose metabolism in the intact mouse. Am J Physiol 221:1738–1745

Hakimi P, Johnson MT, Yang J, Lepage DF, Conlon RA, Kalhan SC, Reshef L, Tilghman SM, Hanson RW (2005) Phosphoenolpyruvate carboxykinase and the critical role of cataplerosis in the control of hepatic metabolism. Nutr Metab 2:33. doi: 10.1186/1743-7075-2-33

Hannaford MC, Goldstein MB, Josse RG, Halperin ML (1982) Role of acidosis in the protein wasting of fasting in the rat and the rabbit. Can J Physiol Pharmacol 60:331–334

Harper AM, Jennett S (1990) Cerebral blood flow and metabolism. Manchester University Press/St. Martin's Press, Manchester/NY

Hawkins RA, Williamson DH, Krebs HA (1971) Ketone-body utilization by adult and suckling rat brain in vivo. Biochem J 122:13–18

Hawkins RA, Miller AL, Cremer JE, Veech RL (1974) Measurement of the rate of glucose utilization by rat brain in vivo. J Neurochem 23:917–923

Heinbecker P (1928) Studies on the metabolism of eskimos. J Biol Chem 80:461–475

Heitmann RN, Dawes DJ, Sensenig SC (1987) Hepatic ketogenesis and peripheral ketone body utilization in the ruminant. J Nutr 117:1174–1180

Hendriks WH, Moughan PJ, Tarttelin MF (1997) Urinary excretion of endogenous nitrogen metabolites in adult domestic cats using a protein-free diet and the regression technique. J Nutr 127: 623–629

Henry CJK, Collingwood A (1998) Allometry of fasting urinary nitrogen loss and basal metabolic rate in homeotherms. Nutr Res 18:49–60

Henry CJK, Rivers JPW, Payne PR (1987) Reduced obligatory nitrogen loss in rats made obese by cafeteria feeding. Nutr Res 7:1243–1251

Hertz L, Dienel GA (2002) Energy metabolism in the brain. In: Dwyer D (ed) Glucose metabolism in the brain. Academic Press Inc., San Diego, pp 1–102

Hill's Pet Nutrition, Inc. (2010) Hills Science diet for cats, adult indoor. http://www.hillspet.com/products/science-diet/sd-feline-adult-indoor-dry.html. Accessed 07 May 2010

Hoenig M (2002) Comparative aspects of diabetes mellitus in dogs and cats. Mol Cell Endocrinol 197:221–229

Hoenig M, Thomaseth K, Waldron M, Ferguson DC (2007) Insulin sensitivity, fat distribution, and adipocytokine response to different diets in lean and obese cats before and after weight loss. Am J Physiol 292:R227–R234

Hoffer LJ (1999) Evaluation of the adaptation to protein restriction in humans. In: El-Khoury AE (ed) Methods for investigation of amino acid and protein metabolism. CRC Press, Boca Raton, pp 83–102

Hoffman RM, Boston RC, Stefanovski D, Kronfeld DS, Harris PA (2003) Obesity and diet affect glucose dynamics and insulin sensitivity in Thoroughbred geldings. J Anim Sci 81:2333–2342

Holliday JA, Steppan SJ (2004) Evolution of hypercarnivory: the effect of specialization on morphological and taxonomic diversity. Paleobiology 30:108–128

Horton TJ, Hill JO (2001) Prolonged fasting significantly changes nutrient oxidation and glucose tolerance after a normal mixed meal. J Appl Physiol 90:155–163

Howe PE, Mattill HA, Hawk PB (1912) Fasting studies. VI. Distribution of nitrogen during a fast of one hundred and seventeen days. J Biol Chem 11:103–127

Iams products for cats (2010) Iams® ProActive Health™ Adult Pate with Chicken & Liver. http://www.iams.com/iams/en_US/jsp/IAMS_Page.jsp?pageID=PL&productID=407#nutritionInformation. Accessed 07 May 2010

Iwaniuk AN (2001) Interspecific variation in sexual dimorphism in brain size in nearctic ground squirrels (*Spermophilus* spp.). Can J Zool 79:759–765

Jeanblain C, Durix A (1985) Ketone body metabolism during pregnancy in the rabbit. Reprod Nutr Dev 25:545–554

Jungas RL, Halperin ML, Brosnan JT (1992) Quantitative analysis of amino acid oxidation and related gluconeogenesis in humans. Physiol Rev 72:419–448

Kaiyala KJ, Prigeon RL, Kahn SE, Woods SC, Porte D Jr, Schwartz MW (1999) Reduced β-cell function contributes to impaired glucose tolerance in dogs made obese by high-fat feeding. Am J Physiol 277:E659–E667

Kamp CW, Mursch DA, Stavinoha WB, Medina MA (1980) Measurement of mouse brain glucose utilization in vivo using [U-^{14}C]glucose. Neurochem Res 5:61–67

Karbowski J (2007) Global and regional brain metabolic scaling and its functional consequences. BMC Biol. doi:10.1186/1741-7007-5-18

Katz J, Tayek JA (1998) Gluconeogenesis and the Cori cycle in 12-, 20-, and 40-h-fasted humans. Am J Physiol 275:E537–E542

Katz J, Tayek JA (1999) Recycling of glucose and determination of the Cori cycle and gluconeogenesis. Am J Physiol 277:E401–E542

Kaufman GA, Kaufman DW (1977) Body composition of the old-field mouse (*Peromyscus polionotus*). J Mammal 58:429–434

Kemppainen J, Aalto S, Fujimoto T, Kalliokoski KK, Langsjo J, Oikonen V, Rinne J, Nuutila P, Knuuti J (2005) High intensity exercise decreases global brain glucose uptake in humans. J Physiol 568:323–332

Kendall PT, Blaza SE, Holme DW (1982) Assessment of endogenous nitrogen output in adult dogs of contrasting size using a protein-free diet. J Nutr 112:1281–1286

Kettelhut IC, Foss MC, Migliorini RH (1980) Glucose homeostasis in a carnivorous animal (cat) and in rats fed a high-protein diet. Am J Physiol 239:R437–R444

Khan JY, Rajakumar RA, McKnight RA, Devaskar UP, Devaskar SU (1999) Developmental regulation of genes mediating murine brain glucose uptake. Am J Physiol 276:R892–R900

Kleiber M (1975) The fire of life: an introduction to animal energetics. Robert E. Krieger Publishing Co., Huntington, NY

Klein S, Wolfe RR (1992) Carbohydrate restriction regulates the adaptive response to fasting. Am J Physiol 262:E631–E636

Kley S, Hoenig M, Glushka J, Jin ES, Burgess SC, Waldron M, Jordan ET, Prestegard JH, Ferguson DC, Wu SX, Olson DE (2009) The impact of obesity, sex, and diet on hepatic glucose production in cats. Am J Physiol 296:R936–R943

Kruska D (1993) Evidence of decrease in brain size in ranch mink, *Mustela vison* f. dom., during subadult postnatal ontogenesis. Brain Behav Evol 41:303–315

Kruska D (2005) On the evolutionary significance of encephalization in some eutherian mammals: effects of adaptive radiation, domestication, and feralization. Brain Behav Evol 65:73–108

Kuzawa CW (1998) Adipose tissue in human infancy and childhood: an evolutionary perspective. Am J Phys Anthropol Suppl 27: 177–209

Leyhausen P (1979) Cat behavior. The predatory and social behavior of domestic and wild cats. Garland STPM Press, New York

Liberg O (1984) Food habits and prey impact by feral and house-based domestic cats in a rural area in Southern Sweden. J Mammal 65:424–432

Likhodii SS, Musa K, Cunnane SC (2002) Breath acetone as a measure of systemic ketosis assessed in a rat model of the ketogenic diet. Clin Chem 48:115–120

Linnet K (1998) Performance of Deming regression analysis in case of misspecified analytical error ratio in method comparison studies. Clin Chem 44:1024–1031

Lobley GE (1992) Control of the metabolic fate of amino acids in ruminants: a review. J Anim Sci 70:3264–3275

Lundbæk K (1948) Metabolic abnormalities in starvation diabetes. Yale J Biol Med 20:533–544

Lusk G (1928) The elements of the science of nutrition, 4th edn. W. B. Saunders Co., Philadelphia (reprinted 1976 by Johnson Reprint Corp., New York)

MacDonald ML, Rogers QR, Morris JG (1984) Nutrition of the domestic cat, a mammalian carnivore. Annu Rev Nutr 4:521–562

Martin GJW, Rand JS (1999) Food intake and blood glucose in normal and diabetic cats fed ad libitum. J Feline Med Surg 1:241–251

Mayntz D, Nielsen VH, Sorensen A, Toft S, Raubenheimer D, Hejlesen C, Simpson SJ (2009) Balancing of protein and lipid intake by a mammalian carnivore, the mink, *Mustela vison*. Anim Behav 77:349–355

McClellan WS, Du Bois EF (1930) Clinical calorimetry. XLV. Prolonged meat diets with a study of kidney function and ketosis. J Biol Chem 87:651–668

Meier PT (1983) Relative brain size within the North American Sciuridae. J Mammal 64:642–647

Miller SA, Allison JB (1958) The dietary nitrogen requirements of the cat. J Nutr 64:493–501

Millward DJ (1995) A protein-stat mechanism for regulation of growth and maintenance of the lean body mass. Nutr Res Rev 8:93–120

Mink JW, Blumenschine RJ, Adams DB (1981) Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. Am J Physiol 241:R203–R212

Mitchell GA, Kassovskabratinova S, Boukaftane Y, Robert MF, Wang SP, Ashmarina L, Lambert M, Lapierre P, Potier E (1995) Medical aspects of ketone body metabolism. Clin Invest Med 18:193–216

Morens C, Sirot V, Scheurink AJW, van Dijk G (2006) Low-carbohydrate diets affect energy balance and fuel homeostasis differentially in lean and obese rats. Am J Physiol 291:R1622–R1629

Morifuji M, Sakai K, Sanbongi C, Sugiura K (2005) Dietary whey protein increases liver and skeletal muscle glycogen levels in exercise-trained rats. Br J Nutr 93:439–445

Morris SM (2002) Regulation of enzymes of the urea cycle and arginine metabolism. Annu Rev Nutr 22:87–105

Morris JG, Trudell J, Pencovic T (1977) Carbohydrate digestion by the domestic cat (*Felis catus*). Br J Nutr 37:365–373

Moundras C, Remesy C, Demigne C (1993) Dietary protein paradox: decrease of amino acid availability induced by high-protein diets. Am J Physiol 264:G1057–G1065

Müller MJ, Paschen U, Seitz HJ (1984) Effect of ketone bodies on glucose production and utilization in the miniature pig. J Clin Invest 74:249–261

Murphy B, Zapol WM, Hochachka PW (1980) Metabolic activities of heart, lung, and brain during diving and recovery in the Weddell seal. J Appl Physiol 48:596–605

National Research Council (1982) Nutrient requirements of mink and foxes. National Academy Press, Washington, DC

National Research Council (2006) Nutrient requirements of dogs and cats. National Academies Press, Washington, DC

Nehlig A, Pereira de Vasconcelos A (1993) Glucose and ketone body utilization by the brain of neonatal rats. Prog Neurobiol 40:163–221

Nielsen K, Kondrup J, Elsner P, Juul A, Jensen ES (1994) Casein and soya-bean protein have different effects on whole body protein turnover at the same nitrogen balance. Br J Nutr 72:69–81

Nordøy ES, Ingebretsen OC, Blix AS (1990) Depressed metabolism and low protein catabolism in fasting grey seal pups. Acta Physiol Scand 139:361–369

Oftedal OT, Alt GL, Widdowson EM, Jakubasz MR (1993) Nutrition and growth of suckling black bears (*Ursus americanus*) during their mothers' winter fast. Br J Nutr 70:59–79

Owen OE, Morgan A, Kemp H, Sullivan J, Herrera M, Cahill GF (1967) Brain metabolism during fasting. J Clin Invest 46:1589–1595

Owen OE, Smalley KJ, D'Alessio DA, Mozzoli MA, Dawson EK (1998) Protein, fat, and carbohydrate requirements during starvation: anaplerosis and cataplerosis. Am J Clin Nutr 68:12–34

Owen OE, Kalhan SC, Hanson RW (2002) The key role of anaplerosis and cataplerosis for citric acid cycle function. J Biol Chem 277:30409–30412

Pagel MD, Harvey PH (1989) Taxonomic differences in the scaling of brain on body weight among mammals. Science 244:1589–1593

Palombo MR, Kohler M, Moya Sola S, Giovinazzo C (2008) Brain versus body mass in endemic ruminant artiodactyls: a case studied of *Myotragus balearicus* and smallest Candiacervus species from Mediterranean islands. Quat Int 182:160–183

Paul AA, Southgate DAT (1978) McCance and Widdowson's The composition of foods. Elsevier/North-Holland Biomedical Press, London

Pazak HE, Bartges JW, Cornelius LC, Scott MA, Gross K, Huber TL (1998) Characterization of serum lipoprotein profiles of healthy, adult cats and idiopathic feline hepatic lipidosis patients. J Nutr 128:2747S–2750S

Pell JM, Bergman EN (1983) Cerebral metabolism of amino acids and glucose in fed and fasted sheep. Am J Physiol 244:E282–E289

Pérez-Barbería F, Gordon I (2005) Gregariousness increases brain size in ungulates. Oecologia 145:41–52

Rand WM, Pellett PL, Young VR (2003) Meta-analysis of nitrogen balance studies for estimating protein requirements in healthy adults. Am J Clin Nutr 77:109–127

Rand JS, Fleeman LM, Farrow HA, Appleton DJ, Lederer R (2004) Canine and feline diabetes mellitus: nature or nurture? J Nutr 134:2072S–2080S

Richard MJ, Holck JT, Beitz DC (1989) Lipogenesis in liver and adipose tissue of the domestic cat (*Felis domestica*). Comp Biochem Phys B 93:561–564

Robinson AM, Williamson DH (1980) Physiological roles of ketone bodies as substrates and signals in mammalian tissues. Physiol Rev 60:143–187

Rogers QR, Morris JG (1979) Essentiality of amino acids for the growing kitten. J Nutr 109:718–723

Rogers QR, Morris JG (2002) Up-regulation of nitrogen catabolic enzymes is not required to readily oxidize excess protein in cats. J Nutr 132:2819–2820

Rogers QR, Morris JG, Freedand RA (1977) Lack of hepatic enzymatic adaptation to low and high levels of dietary protein in the adult cat. Enzyme 22:348–356

Röhrs M, Ebinger P (1999) Verwildert ist nicht gleich wild: Die Hirngewichte verwilderter Haussäugetiere [Feral animals are not really wild: the brain weights of wild domestic animals]. Berl Münch Tierärztl Wochenschr 112:234–238

Rowland N (1981) Glucoregulatory feeding in cats. Physiol Behav 26:901–903

Russell K (2002) Reply to Rogers and Morris. J Nutr 132:2821–2822

Russell K, Lobley GE, Rawlings J, Millward J, Harper EJ (2000) Urea kinetics of a carnivore, *Felis silvestris catus*. Br J Nutr 84:597–604

Russell K, Murgatroyd PR, Batt RM (2002) Net protein oxidation is adapted to dietary protein intake in domestic cats (*Felis silvestris catus*). J Nutr 132:456–460

Russell K, Lobley GE, Millward DJ (2003) Whole-body protein turnover of a carnivore, *Felis silvestris catus*. Br J Nutr 89:29–37

Ryder JW, Kawano Y, Galuska D, Fahlman R, Wallberg-Henriksson H, Charron MJ, Zierath JR (1999) Postexercise glucose uptake and glycogen synthesis in skeletal muscle from GLUT4-deficient mice. FASEB J 13:2246–2256

Sacher GA, Staffeldt EF (1974) Relation of gestation time to brain weight for placental mammals: implications for the theory of vertebrate growth. Am Nat 108:593–615

Sawicka-Kapusta K (1970) Changes in the gross body composition and the caloric value of the common voles during their postnatal development. Acta Theriol 15:67–79

Sawicka-Kapusta K (1974) Changes in the gross body composition and energy value of the bank voles during their postnatal development. Acta Theriol 19:27–54

Scrimshaw NS, Hussein MA, Murray E, Rand WM, Young VR (1972) Protein requirements of man: variations in obligatory urinary and fecal nitrogen losses in young men. J Nutr 102: 1595–1604

Siewert F (2003) Entwicklung der Ernährungsforschung bei der Katze (bis 1975). Dissertation. Tierärztliche Hochschule Hannover, Hannover, Germany

Silva SVPS, Mercer JR (1985) Effect of protein intake on amino acid catabolism and gluconeogenesis by isolated hepatocytes from the cat (*Felis domestica*). Comp Biochem Physiol B 80:603–607

Smadja C, Morin J, Ferre P, Girard J (1990) Initial glucose kinetics and hormonal response to a gastric glucose load in unrestrained post-absorptive and starved rats. Biochem J 270:505–510

Smith M (1926) The minimum endogenous nitrogen metabolism. J Biol Chem 68:15–31

Sokoloff L, Fitzgerald GG, Kaufman EE (1977) Cerebral nutrition and energy metabolism. In: Wurtman RJ, Wurtman JJ (eds) Nutrition and the brain. Raven Press, New York, pp 87–139

Sørensen PG, Petersen IM, Sand O (1995) Activities of carbohydrate and amino acid metabolizing enzymes from liver of mink (*Mustela vison*) and preliminary observations on steady-state kinetics of the enzymes. Comp Biochem Physiol 112B:59–64

Stadie WC, Zapp JA Jr, Lukens FDW (1940) The effect of insulin upon the ketone metabolism of normal and diabetic cats. J Biol Chem 132:423–443

Streja DA, Steiner G, Marliss EB, Vranic M (1977) Turnover and recycling of glucose in man during prolonged fasting. Metabolism 26:1089–1098

Sugden MC (2007) In appreciation of Sir Philip Randle: the glucose–fatty acid cycle. Br J Nutr 97:809–813

Sunehag A, Louie K, Bier JL, Tigas SK, Haymond MW (2002) Hexoneogenesis in the human breast during lactation. J Clin Endocrinol Metab 87:297–301

Swain SD (1992) Energy substrate profiles during fasting in horned larks (*Eremophila alpestris*). Physiol Zool 65:568–582

Tarttelin MF (1991) Specific dietary needs of the cats and some related endocrine and metabolic disorders. Proc Nutr Soc NZ 16:82–92

Taruscio TG, Murphy ME (1995) 3-Methylhistidine excretion by molting and non-molting sparrows. Comp Biochem Physiol A 111:397–403

Tauson A-H, Elnif J, Wamberg S (1997) Nitrogen balance in adult female mink (*Mustela vison*) in response to normal feeding and short-term fasting. Br J Nutr 78:83–96

Tauson AH, Hansen NE, Wamberg S (2001) High versus low protein diets to mink—postprandial plasma urea and creatinine response, osmotic load and pattern of nitrogen and electrolyte excretion. Arch Tierernahr 54:141–158

Thiess S, Becskei C, Tomsa K, Lutz TA, Wanner M (2004) Effects of high carbohydrate and high fat diet on plasma metabolite levels and on iv glucose tolerance test in intact and neutered male cats. J Feline Med Surg 6:207–218

Tonelli J, Kishore P, Lee DE, Hawkins M (2005) The regulation of glucose effectiveness: how glucose modulates its own production. Curr Opin Clin Nutr Metab Care 8:450–456

Uezu N, Yamamoto S, Rikimaru T, Kishi K, Inoue G (1983) Contributions of individual body tissues to nitrogen excretion in adult rats fed protein-deficient diets. J Nutr 113:105–114

US Department of Agriculture, Agricultural Research Service (2009) USDA National Nutrient Database for Standard Reference, Release 22. Nutrient Data Laboratory Home Page, http://www.ars.usda.gov/ba/bhnrc/ndl. Accessed 18 November 2009

VanItallie TB, Nufert TH (2003) Ketones: metabolism's ugly duckling. Nutr Rev 61:327–341

Von Ehrenstein G (1958) Organ weights of TE mice bearing Ehrlich ascites carcinoma growing in the solid form and of C3H mice bearing spontaneous mammary carcinoma. Experientia 14:299–300

Waites GMH, Setchell BP (1964) Effect of local heating on blood flow and metabolism in the testis of the conscious ram. J Reprod Fertil 8:339–349

Wang XM, Tedford RH (2008) How dogs came to run the world. Nat Hist 117:18–23

Waterlow JC (1999) The mysteries of nitrogen balance. Nutr Res Rev 12:25–54

Waterlow JC (2006) Protein turnover. CABI Publishing, Cambridge, MA

Weber JM, Dailly L (1998) Food habits and ranging behaviour of a group of farm cats (*Felis catus*) in a Swiss mountainous area. J Zool 245:234–237

Welle S (1999) Human protein metabolism. Springer, New York

Wester TJ, Weidgraaf K, Ugarte CE, Tavendale MH (2008) Effect of dietary protein level on urea production and protein turnover in the adult cat (*Felis catus*). J Anim Sci 86(E-Suppl 2):213

Wright PA (1995) Nitrogen excretion: three end products, many physiological roles. J Exp Biol 198:273–281

Xiccato G, Bernardini M, Castellini C, Dalle Zotte A, Queaque PI, Trocino A (1999) Effect of postweaning feeding on the performance and energy balance of female rabbits at different physiological states. J Anim Sci 77:416–426

Young VR, Bier DM, Pellett PL (1989) A theoretical basis for increasing current estimates of the amino acid requirements in adult man, with experimental support. Am J Clin Nutr 50:80–92