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Metabolic shifts due to long-term caloric restriction revealed in nonhuman primates

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ABSTRACT

The long-term health benefits of caloric restriction (CR) are well known but the associated molecular mechanisms are poorly understood despite increasing knowledge of transcriptional and related metabolic changes. We report new metabolic insights into long-term CR in nonhuman primates revealed by the holistic inspection of plasma ^{1}H NMR spectroscopic metabolic and lipoprotein profiles. The results revealed attenuation of aging-dependant alterations of lipoprotein and energy metabolism by CR, noted by relative increase in HDL and reduction in VLDL levels. Metabonomic analysis also revealed animals exhibiting distinct metabolic trajectories from aging that correlated with higher insulin sensitivity. The plasma profiles of insulin-sensitive animals were marked by higher levels of gluconate and acetate suggesting a CR-modulated increase in metabolic flux through the pentose-phosphate pathway. The metabonomic findings, particularly those that parallel improved insulin sensitivity, are consistent with diminished adiposity in CR monkeys despite aging. The metabolic profile and the associated pathways are compatible with our previous findings that CR-induced gene transcriptional changes in tissue suggest the critical regulation of peroxisome proliferator-activated receptors as a key mechanism. The metabolic phenotyping provided in this study can be used to define a reference molecular profile of CR-associated health benefits and longevity in symbiotic superorganisms and man.

1. Introduction

Caloric restriction (CR) has long been known to extend maximum lifespan and oppose the development of a broad array of age-associated biological and pathological changes in a diverse range of organisms (Weindruch and Walford, 1988). Accordingly, CR is widely viewed as the most potent dietary means of slowing the aging process. Although the precise molecular mechanisms for this action remain controversial, it is axiomatic that at some level major shifts in energy metabolism are of central importance (Anderson et al., 2008).

Since 1989 we have been testing the ability of adult-onset (8–14 years of age at initiation) CR to retard the aging process in a nonhuman primate model, the rhesus monkey (Ramsey et al., 2000a,b). Rhesus macaques at the Wisconsin National Primate Research Center have an average lifespan of ~27 years and a maximum lifespan of ~40 years. In the present study, we have sought to capture a global view of the metabolic effects of long-term CR in primates using well-validated plasma NMR spectroscopy-based metabolic screening techniques (Nicholson et al., 1995).

Metabonomics provides a powerful approach to study regulatory physiological processes through the quantitative analysis of metabolites in biofluids and tissues of living organisms (Nicholson et al., 1999). This approach efficiently characterizes metabolic phenotypes of mammals *via* data mining of complex metabolic profiles that encapsulate the expression of both host genome and gut microbiome (Martin et al., 2007; Nicholson et al., 2004). The approach was also successfully applied to the diagnosis of pathophysiological states (Brindle et al., 2002) and the pharmacometabonomic prediction of drug metabolism and toxicity from pre-dose metabolic models

Abbreviations: NMR, nuclear magnetic resonance; PCA, principal component analysis; PLS, projection to latent structure; PLS-DA, projection to latent structure discriminant analysis; O-PLS-DA, orthogonal-projection to latent structure discriminant analysis; FAAs, free amino acids; BCAAs, branched-chained amino acids; PPP, pentose-phosphate pathway; TMA, trimethylamine; DMA, dimethylamine; GPC, glycerophosphocholine.

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(Clayton et al., 2006). Recent applications also revealed metabonomics to be particularly well-suited for assessing the effects of nutritional interventions (Rezzi et al., 2007a). As a result of this, we have recently developed the "nutrimetabonomics" concept which opens up new possibilities for characterizing imprinted metabolic signatures associated with dietary patterns and lifestyle (Rezzi et al., 2007b).

Metabonomics has recently been used to study CR-induced metabolic changes in mouse (Selman et al., 2006) and dog models (Richards et al., 2008; Wang et al., 2007). The results indicate that mice responded to acute CR by rapidly switching from lipid biosynthesis to fatty acid catabolism, β-oxidation, and gluconeogenesis, as evidenced by liver and muscle transcripts analyses (Selman et al., 2006). The CR-induced switch in energy metabolism towards energy conservation and gluconeogenesis was sustained by the observed increased plasma levels of lactate, 3-D-hydroxybutyrate, creatine and the glucogenic amino acids, methionine, glutamine, alanine and valine, as revealed by metabonomic analysis (Selman et al., 2006). In addition, the alteration of the plasma lipoprotein profile by CR was reported as a major metabonomic outcome in both mouse and dog models (Richards et al., 2008; Selman et al., 2006). In addition, metabonomics associated long-term CR with modulations of basal energy metabolism *via* decreased urinary excretion of creatine, 1-methylnicotinamide, lactate, acetate and succinate as well as changes of gut microbial activity with significantly higher levels of hippurate, phenylacetylglycine, 4-hydroxyphenylacetate and dimethylamine (Wang et al., 2007).

For the first time, we report a metabonomic investigation of phenotypic changes associated with long-term CR in nonhuman primates. NMR-based metabolic profiling coupled with multivariate statistics were applied to plasma taken from monkeys subjected to CR for 15 years. Metabolic fluctuations differentiating normally aging subjects from CR animals are identified and discussed.

2. Materials and methods

2.1. Experimental design

This trial was conducted at the Wisconsin National Primate Research Center (Madison, WI, USA) and was reviewed and approved by the University of Wisconsin, Graduate School Animal Care and Use Committee. This study of adult (8–14 years of age at study onset) male rhesus monkeys included nine control-fed animals and 11 animals subjected to a 30% reduction in dietary intake (CR). Prior to study initiation, animals were monitored for baseline food intake and body weight (Table 1). Individuals were then equally randomized to either control or CR group based upon age, body weight and baseline food intake levels. CR animals' food allotments were then reduced by 10% per month over a 3-month period to achieve the goal of 30% reduction from individual baseline food intake levels (Colman et al., 1998; Ramsey et al., 2000a). As voluntary food intake levels change with aging, in recent years we have occasionally altered CR animals' food allotments in order to maintain health. At years 2, 9 and 15 of study, fasted morning blood samples were drawn from each animal using potassium oxalate and sodium fluoride as preservatives.

Table 1
Baseline animal characteristics.

	Baseline	
	Control	CR
Age (years)	9.0 ± 0.4	9.0 ± 0.5
Weight (kg)	11.3 ± 0.5	11.3 ± 0.4
Food intake (kcal/day)	730 ± 52	701 ± 43

Values are given as means ± SE; CR, caloric restriction.

2.2. Metabonomic analysis of plasma

Plasma samples (550 μL) were introduced into a 5 mm NMR tube with 50 μL of deuterium oxide (D_2O) used as locking substance and measured on a Bruker Avance 600 MHz spectrometer equipped with an inverse probe and an automatic sample changer (Bruker Biospin, Rheinstetten, Germany) as previously reported (Rezzi et al., 2007b); see Supplementary information (SI). NMR data were prepared and analyzed using unsupervised and supervised pattern recognition methods as previously reported (Rezzi et al., 2007b); see SI. Briefly, after conversion into 22 K data points over the range of δ 0.2–10.0 and removal of residual water resonance (δ 4.5–5.19), the spectra were normalized to a constant total sum of all intensities within the specified range. Multivariate pattern recognition techniques used in this study were based on principal component analysis (PCA) (Wold, 1987) and projection to latent structure (PLS) (Wold et al., 1987) using the software package SIMCA-P+ (version 11.5, Umetrics AB, Umeå, Sweden) and in-house developed MATLAB (The MathWorks Inc., Natick, MA, USA) routines. PCA was first applied to NMR variables (subjected to Pareto scaling, by dividing each variable by the square root of its standard deviation) to detect the presence of inherent similarities between metabolic profiles. Variations between the different plasma metabolic phenotypes were analyzed using scores and loadings plots. Biochemical components (NMR spectral variables) responsible for the differences between individual plasma samples detected in the scores plot can be extracted from the corresponding loadings plot. Additional detailed classification studies were performed using PLS and O-PLS-DA to exclusively focus on the effects of CR on aging (Trygg and Wold, 2002).

2.3. Clinical quantitative measurements of plasma lipids

Triglycerides (TG) were measured using a Wako enzymatic method on a XPAND™ system (Dade Behring, Switzerland). HDL and LDL were determined using the AHDL and ALDL Cholesterol assay systems (Dade Behring, Switzerland). Statistical analysis of the clinical parameters was performed using a two-tailed Mann–Whitney test.

2.4. Insulin sensitivity

Insulin sensitivity was determined by intravenous glucose tolerance testing and analyzed according to the Modified Minimal Model protocol as adapted for rhesus monkeys (Bergman, 1989; Gresl et al., 2003); see SI. Plasma insulin was measured in duplicate by double antibody radioimmunoassay (Linco Research, St. Charles, MO). Total glucose was measured in duplicate with an automated analyzer by use of the glucose oxidase method (Yellow Springs Instruments, Yellow Springs, OH).

2.5. Body composition

Dual-energy X-ray absorptiometry (DXA, Model DPX-L, GE/Lunar Corp., Madison, WI) was used to assess total body fat and lean tissue mass as previously described (Colman et al., 1998, 1999); see SI.

3. Results

Changes in food intake, weight, lean and fat masses for the CR subjects are reported in Table 2. A standard ^{1}H NMR spectrum of rhesus monkeys blood plasma exhibits a set of