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Transcriptome of the subcutaneous adipose tissue in response to oral supplementation of type 2 *Lepr^{db}* obese diabetic mice with niacin-bound chromium

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Rink C, Roy S, Khanna S, Rink T, Bagchi D, Sen CK. Transcriptome of the subcutaneous adipose tissue in response to oral supplementation of type 2 *Lepr^{db}* obese diabetic mice with niacin-bound chromium. *Physiol Genomics* 27: 370–379, 2006. First published August 29, 2006; doi:10.1152/physiolgenomics.00071.2006.— The effects of oral niacin-bound chromium (NBC) supplementation on the subcutaneous fat tissue of type 2 *Lepr^{db}* obese diabetic mice were examined using high-density comprehensive mouse genome (45,101 probe sets) expression arrays. The influence of such supplementation on the plasma cardiovascular risk factors of these mice was also investigated. Supplementation of NBC had no significant effect on age-dependent weight gain in the *Lepr^{db}* obese diabetic mice. However, NBC lowered total cholesterol (TC), TC-to-HDL ratio, LDL cholesterol, and triglyceride levels while increasing HDL cholesterol in the blood plasma. No effect of NBC supplementation was observed on fasting blood glucose levels. Oral glucose tolerance test revealed a significantly improved clearance of blood glucose between 1 and 2 h of glucose challenge in NBC-supplemented mice. Unbiased genome-wide interrogation demonstrated that NBC resulted in the upregulation of muscle-specific gene expression in the fat tissue. Genes encoding proteins involved in glycolysis, muscle contraction, muscle metabolism, and muscle development were specifically upregulated in response to NBC supplementation. Genes in the adipose tissue that were downregulated in response to NBC supplementation included cell death-inducing DNA fragmentation factor (CIDEA) and uncoupling protein-1, which represent key components involved in the thermogenic role of brown adipose tissue and tocopherol transfer protein, the primary carrier of α-tocopherol to adipose tissue. The observation that CIDEA-null mice are resistant to obesity and diabetes suggests that the inhibitory role of NBC on CIDEA expression was favorable. Further studies testing the molecular basis of NBC function and long-term outcomes are warranted.

diabetes; microarray; antioxidant

THE CLASSICAL OBSERVATION in the 1950s that brewer's yeast contained a glucose tolerance factor that prevented diabetes in experimental animals may be viewed as the foundation of current interest in chromium as a nutrient to enhance glucose metabolism (62). Eventually, the glucose tolerance factor emerged as a biologically active form of trivalent chromium that could substantially lower plasma glucose levels in diabetic mice (69). Studies of low-molecular-weight chromium deficiency (30) have led to the identification of this element as a trace essential element involved in the action of insulin (75, 76). Clinical interest in chromium supplementation soared in response to the observation that a patient receiving total parenteral nutrition developed severe indications of diabetes that were reversed on chromium supplementation (22, 30). At present, chromium is added as a standard supplement to total parenteral nutrition (2). The major impediment to the use of orally administered chromium is poor absorption of trivalent chromium in its inorganic form. Trivalent chromium is more available in yeast, and, more recently, organic-bound chromium has been found to be a bioavailable formulation for oral supplementation (18, 79). A combination of chromium and niacin has been reported to significantly decrease total cholesterol and total lipid levels in serum (8).

Obesity and interrelated diabetic disorders represent a major worldwide epidemic (67). The transition from obesity to diabetes is made by a progressive defect in insulin secretion coupled with a gradual rise in insulin resistance. Both insulin resistance and defective insulin secretion appear very prematurely in obese patients, and both worsen similarly toward diabetes. The burden of chronic diseases, such as obesity, type 2 diabetes, and cardiovascular disorders, is expected to increase dramatically. These diseases are a consequence of several factors that include an aging population, changes in demographic composition, and an excess of contemporary lifestyle (42). Normal dietary intake of chromium in humans is often suboptimal (4). Epidemiologic studies suggest that tissue levels of chromium are reduced among diabetic individuals, especially in those with existing cardiovascular diseases, compared with healthy control subjects (12). Physical exercise and pregnancy are also likely to contribute to chromium loss (16, 31). Chromium supplementation holds promise in the management of type 2 diabetes (29, 60). Dietary supplementation of chromium has been postulated to potentially reduce body fat mass and increase lean body mass (74). Supplemental niacin confers diverse benefits with respect to both the quantity and quality of lipid and lipoprotein particles (41, 45). Low doses of niacin are a treatment option for dyslipidemia in patients with type 2 diabetes (26).

In this study, we sought to examine the effects of oral niacin-bound chromium supplementation on the plasma lipid and glucose profile of type 2 diabetic mice. Mice homozygous for the diabetes spontaneous mutation (*Lepr^{db}*; **http://jaxmice.jax.org/strain/000642.html**) were studied. These mice become identifiably obese around 3–4 wk of age. Elevations of plasma insulin begin at 10–14 days and of blood sugar at 4–8 wk. Homozygous mutant mice are polyphagic, polydipsic, and

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polyuric. Exogenous insulin fails to control blood glucose levels, and gluconeogenic enzyme activity increases. Wound healing is delayed, and metabolic efficiency is increased. The influence of niacin-bound chromium supplementation on the transcriptome of subcutaneous fat of these mice was examined using high-density, comprehensive, whole mouse genome expression arrays.

MATERIALS AND METHODS

Animals, supplement, and supplementation protocol. Male obese *Lepr^{db}* diabetic mice (n = 14, BKS.Cg-*m* +/+ *Lepr^{db}*/J, stock 000642; Jackson Laboratories, Bar Harbor, ME) were received at 8 wk of age and randomly divided into the following groups: supplemented with niacin-bound chromium (n = 7; NBC) and placebo control (n = 7; PBO). Mice were maintained under standard housing conditions at 22 ± 2°C with 12:12-h light-dark cycles. With an allowance of 2 wk for environmental and trainer handling acclimation, mice began a supplementation regimen of either NBC suspended in water at a concentration of 10 mg/kg body wt or a matching volume of water that served as PBO at 10 wk of age. NBC used for the supplementation in this study was identical to the preparation examined in human studies (18, 51). NBC containing 10% elemental chromium in a fine-mesh powder (CM-100M) was obtained from InterHealth Nutraceuticals (Benicia, CA). The dose of NBC to supplement was determined on the basis of two factors: *1*) a small pilot study with a smaller dose of 2 $mg \cdot kg^{-1} \cdot day^{-1}$ and shorter supplementation time of 4 wk revealed just less than statistically significant changes in phenotypic outcomes, and *2*) a review of literature (1, 3, 5, 36) indicating that human chromium consumption that exceeds the adequate daily dietary intake is safe. Consistently, we saw no signs of overt toxicity in this in vivo study.

Throughout the course of treatment, all mice had access to standard mouse chow (Harlan) and water ad libitum, with the exception of 12 h before blood glucose and lipid profiling and 6 h before the oral glucose tolerance test. Oral gavaging was implemented using a 22G feeding needle from Popper and Sons (New Hyde Park, NY). Mice were gavaged 5 days/wk for a period of 10 wk. The body weight of mice, by which the dose of NBC was determined, was obtained at baseline (i.e., start of study) and every 2 wk throughout the course of study. Total volume of the aqueous supplement throughout the study averaged 146 ± 20 μl. Following 10 wk of supplementation, mice were euthanized, and subcutaneous fat was isolated for RNA extraction and microarray analysis. All animal protocols were approved by the Institutional Laboratory Animal Care and Use Committee of the Ohio State University, Columbus, OH.

Blood glucose and lipid profiling. Blood glucose and lipid panel profiling was performed at baseline before any supplementation (*week 0*) and at week 6. Following a 12-h fast, mice were briefly anesthetized with isoflurane to acquire 100 μl of blood via the retroorbital vein. The blood samples were subjected to glucose and lipid profile analyses using a clinical CardioChek analyzer (Polymer Technology Systems, Indianapolis, IN). Parameters analyzed include blood glucose, total cholesterol, HDL cholesterol, triglycerides, LDL, and the total cholesterol-to-HDL cholesterol ratio.

Oral glucose tolerance test. On the eighth week of supplementation, all mice were subjected to an oral glucose tolerance test (OGTT). As a part of this test, blood was drawn after 6 h of chow withdrawal. Baseline (t = 0) blood glucose measurements were recorded using the CardioChek analyzer. Mice were then challenged with 1.5 mg/g body wt of D-glucose (Sigma) dissolved in water, and blood glucose levels were measured at 30, 60, and 120 min after glucose challenge.

GeneChip probe array analysis. Total RNA was isolated from subcutaneous fat by grinding the tissue under liquid nitrogen as previously described (32, 55, 57, 58). RNA was purified using the RNeasy kit (Qiagen), and quality was checked using a Bio-Rad Experion RNA HighSens lab chip. Targets for microarray hybridization were prepared according to a previously described protocol (55). Briefly, samples were hybridized for 16 h at 45°C to GeneChip Test-2 arrays to assess preparation quality. On verification of sample quality, the targets were hybridized to Affymetrix mouse genome arrays (430 v2.0) under the conditions listed above for the Test-2 arrays. The arrays were washed, stained with streptavidin-phycoerythrin, and then were scanned with the GeneArray scanner. All procedures related to microarray analyses were conducted in our own laboratory and facilities.

Microarray data analysis. Raw data were collected and analyzed using the Affymetrix Microarray Suite 5.0 (MAS) and Data Mining Tool 2.0 (DMT) software as described previously (56, 57, 73). Additional processing of data was performed using the dChip software (39). A detailed analysis scheme has been illustrated (see Fig. 4). Absolute analysis was utilized to identify differentially expressed genes (57). The t-test was performed using DMT on absolute files generated from MAS. Genes that significantly ($P < 0.05$) changed (increased or decreased) in the supplemented group compared with the control group were selected. Next, the dChip (v 1.3, Harvard University) software was used to further filter genes using the following criteria: *1*) fold change >1.2; *2*) t-test, $P < 0.05$; and *3*) present call in all experimental (NBC) samples for upregulated genes, and present call in all baseline (PBO) samples for downregulated genes. For data visualization, genes filtered using the statistical (t-test) approach were subjected to hierarchical clustering using the dChip (v1.3) software (56, 57, 73).

Real-time RT-PCR analysis. Expression levels of candidate genes (calsequestrin-1, CASQ1; tropomyosin-1, TPM1; enolase-3, ENO3; glucose phosphate isomerase, GPI1; uncoupling protein-1, UCP1; cell death-inducing DNA fragmentation factor, CIDEA; tocopherol transfer protein, TTP) and GAPDH mRNA were independently determined using real-time RT-PCR as described previously (57). In brief, total RNA (5 μg) was reverse transcribed into cDNA using oligo-dT primer and Superscript II. RT-generated DNA was quantified by real-time PCR assay using double-stranded DNA binding dye SYBER Green-I as described previously (57). Individual gene primer sequences are listed in Table 1.

Data presentation and analysis. Data are shown as means ± SD. Differences between means were tested using Student's t-test or ANOVA, as appropriate. Difference between means was considered significant at $P < 0.05$.

RESULTS

Mice homozygous for the diabetes spontaneous mutation (*Lepr^{db}*) become identifiably obese around 3–4 wk of age.

Table 1. *Primer sequences used for real-time PCR analyses*

mRNA	Primer Sequence 5′ to 3′
CASQ1	CTTCGACAAGGTGGCAA
	CAGTTTCCTCAGGGTTGATCTCCT
TPM1	ACCGGAGCAAGCAGCTGGAA
	GCACGATCCAACTCCTCCTCAA
ENO3	CCTGTGCCTGCCTTTAATGTGA
	CGTCCTTCCCATACTTGGCCTT
GPI1	GAAACCGCCCGACCAACTCTATT
	CCTCCAGCTCCGGCTCAATT
UCP1	CCGCTACACGGGGACCTACAAT
	CCGGCAACAAGAGCTGACAGTAA
CIDEA	GTCATCACAACTGGCCTGGTTACG
	GCCTGTATAGGTCGAAGGTGACTCT
TTP	CTATTATAAATGGCGAGCAGAATGC
	TTTTGGGTCCCAGTATGCAATT

CASQ1, calsequestrin 1; TPM1, tropomyosin 1; ENO3, enolase 3; GPI1, glucose phosphate isomerase 1; UCP1, uncoupling protein 1; CIDEA, cell death-inducing DNA fragmentation factor; TTP, tocopherol transfer protein.

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Elevations of plasma insulin begin at 10–14 days age and of blood sugar at 4–8 wk. We therefore chose to start our study at 10 wk of age. Homozygous mutant mice are polyphagic, polydipsic, and polyuric. A number of diabetes-related features are observed on the C57BLKS background, including an uncontrolled rise in blood sugar, severe depletion of the insulin-producing β-cells of the pancreatic islets, and death by 10 mo of age. Exogenous insulin fails to control blood glucose levels, and gluconeogenic enzyme activity increases (13, 14). Oral supplementation of NBC to the obese diabetic mice for 10 wk had no significant effect on body weight compared with PBO-fed controls (Fig. 1). While subjected to gavaging, animals were observed for early removal criteria such as mutilation, extreme lethargy, guarding, and weight loss of greater than an average of 10% per week. On the basis of these early removal criteria, no animals had to be pulled from the study. Furthermore, chow and water consumption were monitored daily during gavaging, and no discernable difference was observed in the consumption between experimental and control groups (data not shown). There were no overt differences in phenotypic body composition observed between NBC-supplemented and PBO mice following the 10 wk of oral gavaging.

Obesity is associated with hypertension and a greater risk for cardiovascular disease as a result of elevated levels of circulating total cholesterol, LDL, and triglycerides, which are known to induce atherosclerosis (28). While NBC supplementation had no significant effect on age-dependent weight gain in the *Lepr^{db}* obese diabetic mice, there were significant differences in the blood lipid profiles between NBC- and PBO-supplemented groups (Fig. 2). *Lepr^{db}* mice supplemented with NBC had significantly (20%) lower total cholesterol, higher (25%) HDL cholesterol, lower (54%) LDL cholesterol, and lower (43%) triglyceride levels compared with PBO-gavaged mice following 6 wk of supplementation. The total cholesterol (TC)-to-HDL ratio (TC:HDL) is frequently used to assess the risk of heart disease (63, 70). Following 6 wk of oral supplementation, mice treated with NBC had a significantly lower (37%) TC:HDL ratio compared with mice in the PBO group orally gavaged with a matching volume of water.

In the present study, no difference was observed in fasting blood glucose levels of supplemented and control *Lepr^{db}* mice following 6 wk of NBC supplementation (Fig. 3*A*). Eight weeks into the study, an OGTT was implemented to elucidate the effects of NBC supplementation on carbohydrate metabolism in obese diabetic mice. After a glucose challenge of 1.5 mg/g body wt, mice that were supplemented with NBC had no significant difference in blood glucose challenge response (0 min) or the rate of clearance at 30 and 60 min compared with PBO-supplemented mice (Fig. 3*B*). From 60 to 120 min, however, there was a decline in the rate of blood glucose clearance, as NBC-supplemented mice had a significantly greater reduction in the percent change of blood glucose compared with PBO-gavaged control mice.

This work represents the first to investigate the genome-wide effects of chromium supplementation in a rodent model of obesity and diabetes. While no overt differences in body weight were observed between NBC-supplemented *Lepr^{db}* obese diabetic mice and matched PBO-supplemented controls, the apparent changes in the lipid profile and response to glucose challenge led us to examine the transcriptome of the subcutaneous adipose tissue. To achieve a comprehensive analysis of adipose tissue gene expression profiling after 10 wk of either NBC or PBO supplementation, high-density DNA microarray analysis was performed. The overall objective of the data mining approach was the elucidation of candidate genes within the adipose tissue that were sensitive to oral NBC

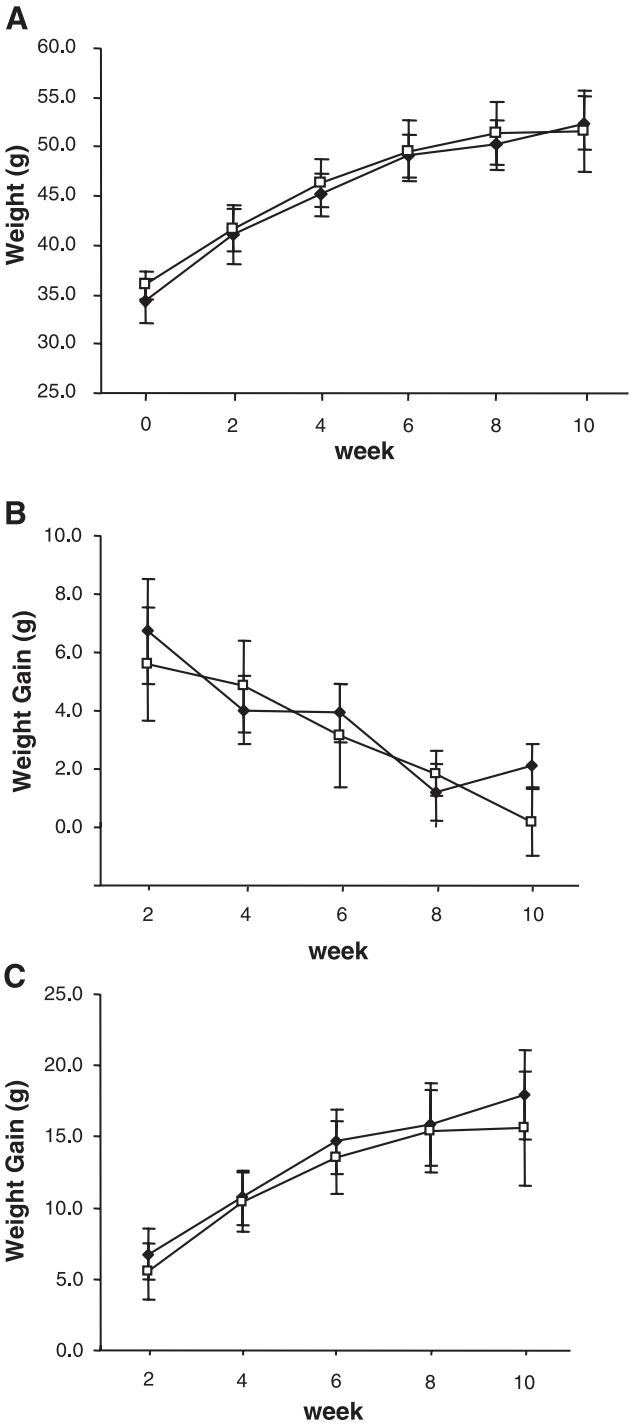

Fig. 1. Body weight in response to niacin-bound chromium (NBC) supplementation. Two groups of obese diabetic mice ($n = 7$) were orally gavaged 5 days/wk with either NBC (10 mg/kg body wt, □) or a matched volume of water (◆). Individual mouse weight was recorded every 2 wk. Data represent mean weight of mice over time (*A*), mean weekly weight gained (*B*), and mean cumulative weight gained for duration of supplementation (*C*) ± SD.

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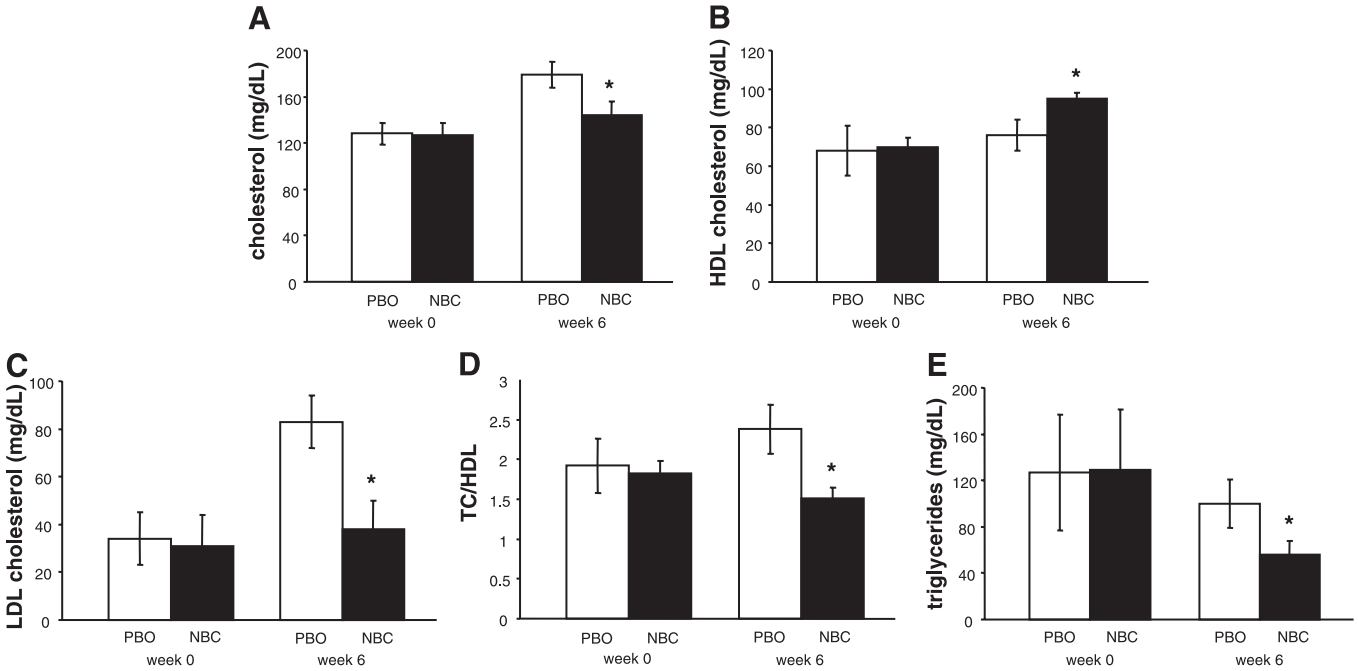

Fig. 2. Plasma lipid profile. Lipid profile of the blood plasma was analyzed at baseline (*week 0*) and following 6 wk of oral gavaging with either NBC (10 mg/kg, solid bars) or a matching volume of placebo water (PBO; open bars). Blood (50 μl) collected from the retroorbital vein was used to quantify the following parameters: cholesterol (*A*), HDL cholesterol (*B*), LDL cholesterol (*C*), total cholesterol (TC)-to-HDL ratio (*D*), and triglycerides (*E*). *$P < 0.0005$.

supplementation. Figure 4 illustrates the data analysis scheme adopted. This design is consistent with previous studies by our laboratory (57, 58). Of the 45,101 probe sets analyzed, only a very small and consistent subset was up- or downregulated in response to NBC supplementation. This reflects a specific effect of oral NBC and argues against a genome-wide perturbation caused by the nutritional supplement. The dChip software enabled graphic visualization of the tight consistency of the NBC-sensitive genes in the subcutaneous adipose tissue (Fig. 5). A sample of NBC-sensitive upregulated (Table 2) and downregulated (Table 3) genes are listed.

Validation of DNA microarray data was carried out by real-time PCR analysis of select candidate genes. Genes of interest were chosen on the basis of a significant fold change $\geq$1.2. While accurately predicting the direction of the fold change, we have previously reported that DNA microarray analyses tend to underestimate the degree of gene induction compared with real-time PCR analysis (57, 58). This property is evident in the present study's validation of both upregulated and downregulated genes by real-time PCR (Figs. 6 and 7). Dissociation curves for all genes verified by real-time PCR revealed no primer-dimer formation or contaminating adducts (data not shown). Interestingly, a large number of genes upregulated by a 1.6-fold change or greater in adipose tissue were myogenic in nature. NBC supplementation upregulated the expression of the following genes: CASQ1, a Ca^{2+} mediator for muscle contraction; TPM1, a myosin regulator; and both ENO3 and GPI1, key enzymes necessary for glycolysis. Findings from quantitative real-time PCR assay were consistent with the results of the DNA microarray analysis (Fig. 6). Real-time PCR validation of the downregulated genes CIDEA, UCP1, and TTP also was consistent with the DNA microarray results (Fig. 7).

DISCUSSION

Obesity is an epidemic disease associated with numerous cardiovascular risk factors including diabetes mellitus and dyslipidemia. Both insulin resistance and defective insulin secretion appear very prematurely in obese patients, and both worsen similarly toward diabetes (23). There is scant evidence suggesting that supplemental chromium may cause weight loss (74). Recently, a meta-analysis was performed to assess the evidence of chromium supplements for reducing body weight. The results indicated a relatively small effect of chromium picolinate compared with placebo for reducing body weight (49). This outcome is not surprising, because it is well accepted that effective strategies of weight loss require management approaches in a combined approach of dietary therapy and physical activity by using behavioral interventions (37). Thus controlled studies testing the effect of chromium alone may not generate overt changes in body weight outcomes. Interestingly, NBC supplemented daily over 2 mo by African American women undergoing a modest dietary and exercise regimen influenced weight loss and body composition (18). Such encouraging effects may be attributed to the combinatorial therapeutic approach adopted including supplementation, diet, and exercise. Although the current experimental design did not affect body weight, it was powerful for examining the specific effects of NBC by itself in vivo.

A higher risk of future cardiovascular disease is seen in patients who have diabetes with or without preexisting cardiovascular disease than in nondiabetic subjects with preexisting cardiovascular disease. Recent guidelines for treating patients with diabetes categorize the disorder as a coronary heart disease equivalent and urge aggressive treatment of modifiable risk factors, such as plasma levels of LDL cholesterol. Li-

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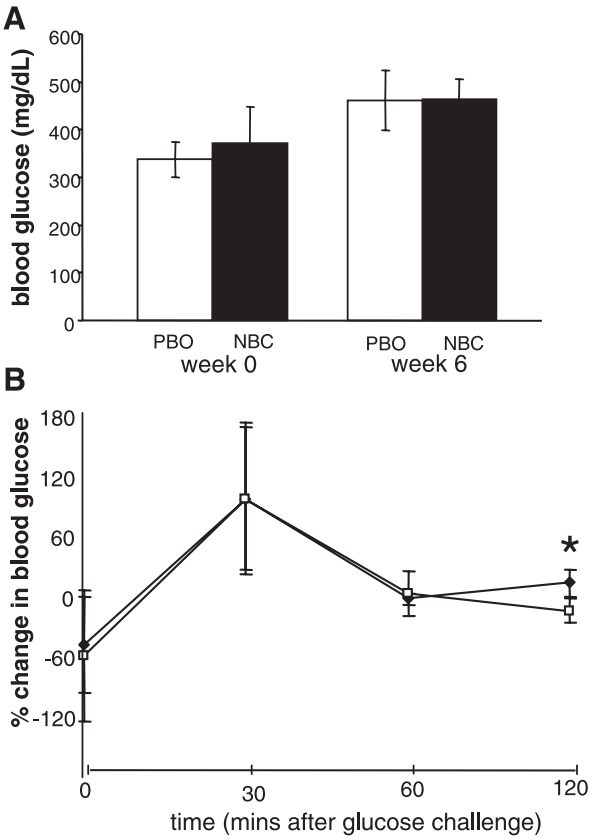

Fig. 3. Blood glucose and glucose clearance. *A*: blood glucose was determined at baseline (*week 0*) and following 6 wk of oral gavaging with either NBC (10 mg/kg, solid bars) or a matching volume of placebo water (PBO; open bars). *B*: after 8 wk of supplementation, mice administered PBO and NBC (10 mg/kg) were fasted for 6 h, after which they were orally gavaged with an aqueous solution of glucose (1.5 mg glucose/g body wt). Blood samples were collected at administration of glucose challenge and 30, 60, and 120 min thereafter to compare the blood glucose clearance rate between NBC-supplemented (□) and PBO controls (◆). Data represent mean %change of blood glucose between time points. Blood glucose value before fasting was used for a baseline reference point. *$P \leq 0.005$: significant difference between PBO and NBC-supplemented groups.

poprotein abnormalities may accompany insulin resistance (54). Both the American Diabetes Association and the National Cholesterol Education Program consider type 2 diabetes mellitus to be a coronary artery disease risk equivalent and thus suggest that patients with either diabetes or coronary artery disease should have their plasma levels of LDL cholesterol lowered (27). Obese diabetic mice are known to have significantly higher levels of TC, LDL, and triglycerides (34, 47). Metabolic syndrome as defined by the American Heart Association is characterized by a group of metabolic risk factors in any one individual that include, but are not limited to, abdominal obesity, high triglycerides, low HDL cholesterol, high LDL cholesterol, and insulin resistance or glucose intolerance (25). The mice used in the current study represent a powerful tool to model the metabolic syndrome (19). NBC supplementation clearly improved the lipid profile of the blood plasma. Lower TC, higher HDL cholesterol, lower LDL cholesterol, and lower triglyceride levels in response to NBC were highly significant findings. These findings are consistent with the literature on humans and experimental rodents. Twelve weeks of chromium supplementation has been observed to lower levels of blood plasma LDL cholesterol and lower levels of TC as well as lower HDL cholesterol and triglyceride in rats (66). Chromium has been implicated as a cofactor in the maintenance of normal lipid and carbohydrate metabolism. In humans, high dietary intake of chromium over a long period lowered TC, triglyceride, and hemoglobin A(1C) in blood (64). Two months of chromium supplementation resulted in a clinically useful increase in HDL cholesterol levels in men taking beta-blockers (53). In a double-blind crossover study with humans, it was noted that levels of TC, LDL cholesterol, and apolipoprotein B (the principal protein of LDL) decreased significantly while the subjects were supplemented with chromium. The concentration of apolipoprotein A-I, the principal protein of HDL fraction, increased during the treatment. The HDL cholesterol level was elevated slightly, but not significantly, during chromium supplementation. In contrast, only apolipoprotein B was altered significantly during supplementation with the placebo. In a recent double-blind, placebo-controlled trial with obese diabetic patients, it was reported that, following 6 mo of NBC supplementation, significant improvements were found in LDL levels, total-to-HDL cholesterol ratios, and TC levels (33). These observations support the assertion that dietary chromium supplementation is efficacious in lowering blood lipids in humans (50). In this study, although NBC supplementation had a significant influence on

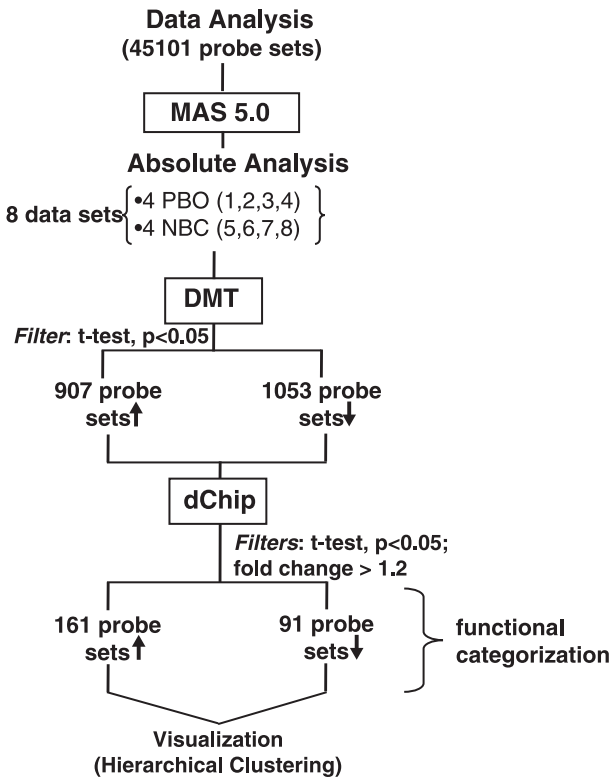

Fig. 4. GeneChip data analysis scheme. Data processing was primarily performed using Microarray Suite 5.0 (MAS) and Data Mining Tool v2.0 (DMT) softwares. Additional data filtration was performed using dChip with the following criteria: *1*) fold change >1.2; *2*) *t*-test, $P < 0.05$; and *3*) present call in all experimental (NBC supplemented) samples for upregulated genes, vice versa present call in all PBO samples for downregulated genes. Details of software and other resources for data analysis are provided in MATERIALS AND METHODS. ↑, Increases; ↓, decreases in response to NBC supplementation (10 mg/kg body wt).

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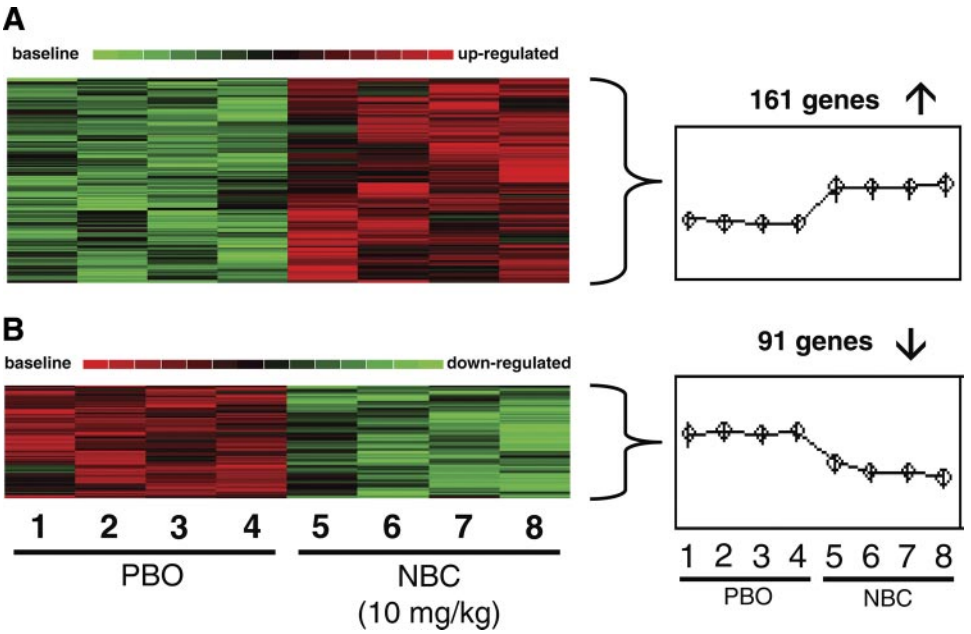

Fig. 5. Cluster images illustrating genes sensitive to NBC in the subcutaneous fat tissue. Student's *t*-test was performed on data from NBC- and PBO-supplemented groups in subcutaneous fat. The genes that significantly ($P < 0.05$) changed between the 2 groups compared were selected and subjected to hierarchical clustering using dChip software as described in Fig. 4. Upregulated (*A*) and downregulated genes (*B*) in NBC-supplemented compared with PBO (baseline) group.

lipid profile of the blood plasma, blood glucose was not influenced. The *Lepr^{db}* mouse model is characterized by an uncontrolled rise in blood sugar, severe depletion of the insulin-producing β-cells of the pancreatic islets, and death by 10 mo of age. Exogenous insulin fails to control blood glucose levels, and gluconeogenic enzyme activity increases. Under such severe genetic conditions, it is understandable that the dietary supplement NBC was not effective in changing blood glucose levels. The marginal effect of NBC on OGTT suggests improved clearance of glucose in treated mice. This indication is consistent with the current literature demonstrating that supplemental chromium may ameliorate hyperglycemia after a glucose load (43). Results of a human study show that dietary chromium supplementation raised HDL cholesterol and improved insulin sensitivity in those with evidence of insulin resistance but normal glucose tolerance (52).

Adipose cells and skeletal myoblasts are derived from a common mesodermal stem cell, indicating that both cells have a closer relationship in the developmental lineage than the other somatic cells. Recently, it has been demonstrated that cells from adipose tissue exhibit mesenchymal plasticity such that they display skeletal myogenic potential (38). This is consistent with the observation that preadipocytes are capable of myogenic differentiation (61). Adipose tissue is known to contain pluripotent mesenchymal cells that are capable of differentiating along the myogenic lineage (78). Adipose tissue, like bone marrow, is derived from the embryonic mesenchyme and contains a stroma. Stem cell population within the adipose stromal compartment, also known as processed lipoaspirate cells, may differentiate toward a myogenic lineage (81). Indeed, a myogenic fate of adipose stromal cell-derived common pluripotent stem cells has been reported (10). MyoD exerts a master transcriptional control over the myogenic differentiation cascade. Studies of organotypic cultures of fat tissue and a long-term culture of in vitro differentiated adipocytes demonstrate that MyoD provokes morphological changes in mature adipocytes that can be summarized as loss of fat content, acquisition of a fusiform shape, and eventual fusion with committed neighbor cells. In vivo, MyoD gene transfer into rat fat pads demonstrated that, while structural proteins of

Table 2. *List of genes in subcutaneous fat that were upregulated in response to oral supplementation of niacin-bound chromium*

Gene Name	FC Average	FC SD	Function
Enolase 3	7.6	3.6	glycolysis
Calsequestrin 1	4.6	2.0	regulation of muscle contraction
Actin	4.4	1.7	muscle contraction
Tropomyosin 1	3.5	1.3	muscle development
GTPase-activating RANGAP domain-like 1	3.3	1.0	GTPase activator activity
Protease inhibitor 16	3.1	1.1	peptidase activity
Aspartate beta hydroxylase	2.9	0.6	macromolecule metabolism
Endothelin receptor type B	2.8	0.8	G-coupled protein receptor activity
Talin 1	2.8	0.4	actin binding
Sarcalumenin	2.4	0.7	calcium ion binding
Thromobospondin, type 1, domain 2	2.3	0.5	protein amino acid phosphorylation
Nebulin-related anchoring protein	2.1	0.3	ion transport
Glucose phosphate isomerase 1	1.6	0.3	glycolysis

FC, fold change; SD, standard deviation.

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Table 3. *List of genes in subcutaneous fat that were downregulated in response to oral supplementation of niacin-bound chromium*

Gene Name	FC Average	FC SD	Function
DDHD domain containing 1	2.7	0.30	lipid catabolism
Uncoupling protein 1	2.7	1.00	mitochondrial transport
Neuroepithelial cell transforming gene 1	2.2	0.70	guanyl-nucleotide exchange factor activity
Cell death-inducing DNA fragmentation factor	2.1	0.02	lipid metabolism
Betacellulin	1.9	0.49	growth factor activity
Phosphoenolpyruvate carboxykinase 1	1.8	0.04	gluconeogenesis
Tocopherol transfer protein	1.4	0.13	vitamin E transport

muscle lineage were expressed, they coexisted with specific adipocyte proteins (35). Unbiased genome-wide interrogation of the effect of dietary NBC on the transcriptome of the subcutaneous adipose tissue posits that the supplement resulted in the upregulation of muscle-specific gene expression. Whether NBC triggered a myogenic response in the fat tissue is an interesting, testable hypothesis that resulted from our microarray studies. NBC supplementation had an overall positive impact on the fat genome, where it induced many more genes than it repressed. Genes encoding proteins involved in glycolysis, muscle contraction, muscle metabolism, and muscle development were specifically upregulated in response to NBC supplementation. Expression of muscle-specific genes in the fat tissue is known, over time, to diminish the fat content of the tissue. This transdifferentiation process is well tolerated by the fully differentiated and mature adipocytes (35).

Enolase, or ENO3, was the most sensitive among all genes induced in the fat tissue in response to NBC supplementation. Enolase is a dimeric glycolytic enzyme exhibiting tissue-specific isoforms. The enzyme catalyzes the interconversion of 2-phosphoglycerate and phospho*enol*pyruvate. In adult human muscle, >90% of enolase activity is accounted for by the β-enolase subunit, the protein product of the ENO3 gene (17). The transcription of ENO3 is regulated by an intronic muscle-specific enhancer that binds myocyte-specific enhancer factor-2 proteins and ubiquitous G-rich box-binding factors (21). Deficiency in β-enolase leads to myopathy (17). In the order of the magnitude of change, the next NBC-sensitive gene in the fat tissue was calsequestrin. Calsequestrin is by far the most abundant Ca^{2+}-binding protein in the sarcoplasmic reticulum of skeletal and cardiac muscle. It allows the Ca^{2+} required for contraction to be stored at total concentrations of up to 20 mM, while the free Ca^{2+} concentration remains at ~1 mM. This storage capacity confers on muscle the ability to contract frequently with minimal run-down in tension (7). Chromium is known to induce the plasmalemmal Ca^{2+}-ATPase in smooth muscle cells (40), raising the possibility that bioactive chromium modulates calcium metabolism. Indeed, chromium has been shown to reduce intracellular Ca^{2+} concentration loads in vascular smooth muscle cells and thereby reduce peripheral

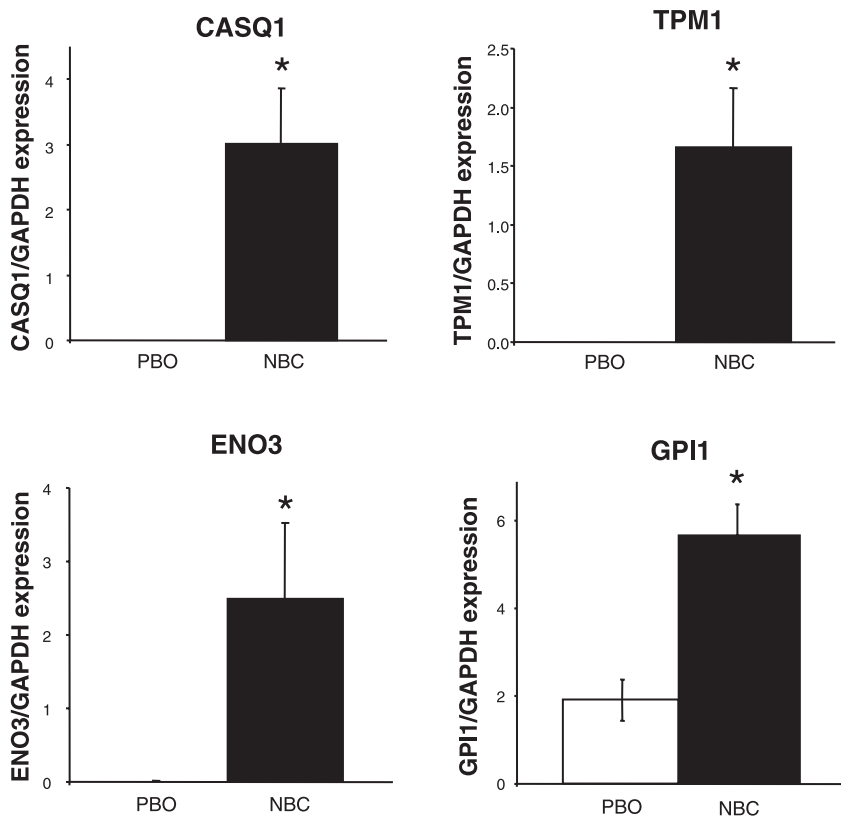

Fig. 6. Quantitative expression analyses of genes upregulated in response to NBC supplementation. CASQ1, calsequestrin-1; TPM1, tropomyosin-1; ENO3, enolase-3; GPI1, glucose phosphate isomerase-1. *$P < 0.05$.

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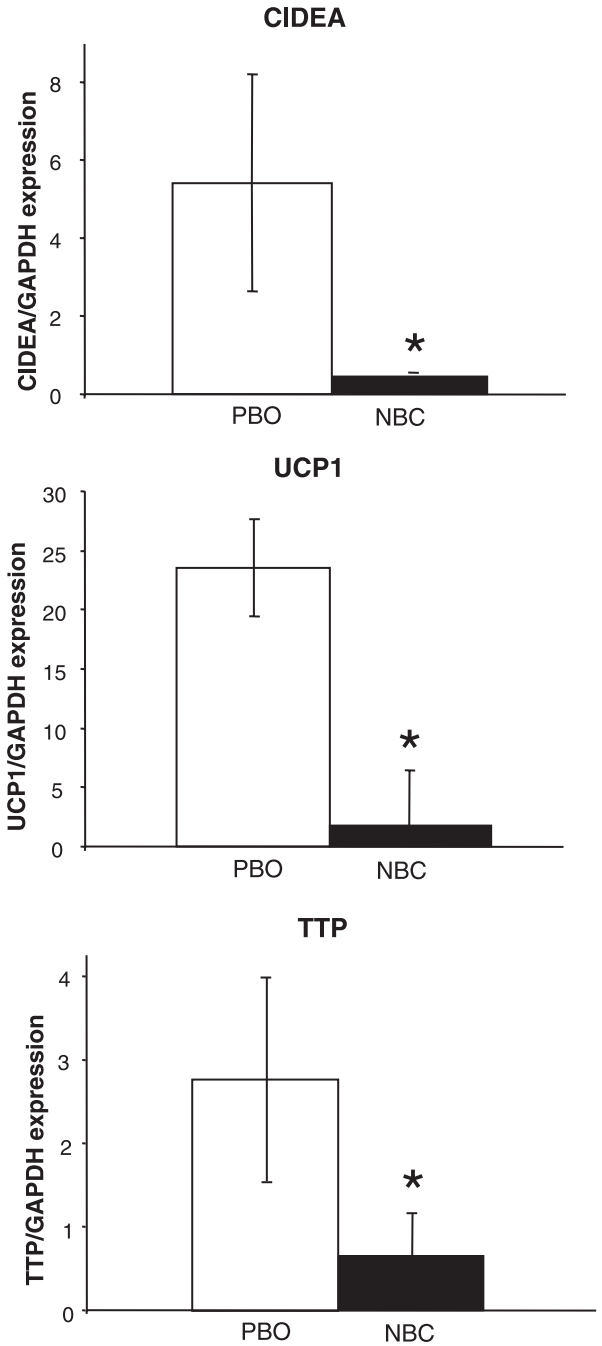

Fig. 7. Quantitative expression analyses of genes downregulated in response to NBC supplementation. CIDEA, cell death-inducing DNA fragmentation factor; UCP1, uncoupling protein-1; TTP, tocopherol transfer protein. *$P <$ 0.05.

vascular resistance in insulin-resistant states (44). Some of the other most-affected genes that were induced in response to NBC supplementation in the fat tissue include actin and tropomyosin-1. Actin plays a dynamic role in muscle contraction and many cellular motility events that occur when the motor domain of myosin uses the energy of ATP hydrolysis to move along the actin filament (77). Tropomyosin, in association with the troponin complex, plays a central role in the Ca^{2+}-dependent regulation of striated muscle contraction. Skeletal isoforms are composed of two types of subunits, α and β (59). TPM1 encodes tropomyosin-1 or α-tropomyosin (20). Finally, glucose phosphate isomerase (GPI) is another glycolytic enzyme that is responsible for the conversion of glucose-6-phosphate to fructose-6-phosphate. It has been reported that glycolytic genes, such as GPI and ENO3, are downregulated in the visceral adipose tissue of morbidly obese patients compared with nonobese counterparts (6). Increased GPI expression in subcutaneous fat in response to NBC supplementation is consistent with a myogenic induction of adipose tissue, as GPI is highly expressed in skeletal muscle (9). Genes in the adipose tissue that were downregulated in response to NBC supplementation included CIDEA and UCP1, which represent key components involved in the thermogenic role of brown adipose tissue (15, 80). The UCP1 gene is uniquely expressed in brown adipose tissue and uncouples ATP generation and dissipates the energy as heat (11). White adipose tissue contributes to UCP1-independent thermogenesis (24). Brown adipose tissue expresses the thermogenic UCP1. UCP1 is positively regulated by peroxisome proliferator-activated receptor (PPAR) agonists and retinoids through the activation of the heterodimers PPAR/retinoid X receptor (RXR) and retinoic acid receptor (RAR)/RXR and binding to specific elements in the UCP1 enhancer (68). UCP1 and adipocyte-specific genes are downregulated by extracellular-regulated kinases and p38 mitogen-activated protein kinase-dependent pathways (72). The expression of UCP1 seems to be tightly related to adipocyte growth (71). Cell death-inducing DNA fragmentation factor-α (DFFA)-like effector A (CIDEA) belongs to a family of proapoptotic proteins that has five known members in humans and mice. CIDEA regulates energy balance and adiposity. CIDEA-null mice are resistant to obesity and diabetes (80). CIDEA mRNA is expressed in white human fat cells and in brown mouse adipocytes. Lowering of CIDEA gene expression by the RNA interference approach stimulates lipolysis (48). Both UCP1 and CIDEA are regulated by similar pathways because both genes are downregulated by TNFα (48, 72). The pathways involved in NBC-dependent downregulation of UCP1 and CIDEA remain unknown. TTP is the primary carrier of α-tocopherol from the liver to peripheral organs including adipose tissue (65). Expression of TTP in subcutaneous fat is high, as adipose tissue serves as a reservoir for >90% of the lipid-soluble vitamin stored in the body (46). α-Tocopherol is a potent lipid-phase antioxidant. Downregulation of TTP expression is expected to lower the levels of α-tocopherol in adipocytes compromising their lipid-phase antioxidant defense mechanism.

In summary, this study presents direct evidence establishing that dietary supplementation of NBC is effective in improving plasma lipid profile in obese diabetic mice. NBC supplementation did not influence age-dependent gain in body weight of the *Lepr^{db}* mice. Unbiased genome-wide interrogation of the transcriptome of the subcutaneous adipose tissue led to the hypothesis that NBC triggered a myogenic response in the fat tissue. Genes encoding proteins involved in glycolysis, muscle contraction, muscle metabolism, and muscle development were specifically upregulated in response to NBC supplementation. Genes in adipose tissue that were downregulated in response to NBC supplementation were fewer than those that were induced. Genes in this category included CIDEA and UCP1, which are known to be regulated by common pathways. The observation that CIDEA-null mice are resistant to obesity and

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diabetes indicates that the inhibitory role of NBC on CIDEA expression is favorable. Further studies testing the molecular basis of NBC function and long-term outcomes in the treatment of type 2 diabetes are warranted.

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