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Dietary Intervention with Vitamin D, Calcium and Whey Protein Reduced Fat Mass and Increased Lean Mass in Rats

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Abstract

The aim of the current study is to determine the effects and the mechanisms of inclusion of dietary whey protein, high calcium and high vitamin D intake with either a high sucrose or high fat base diets on body composition of rodents. Male Wistar rats were assigned to either no whey protein, suboptimal calcium (0.25%) and vitamin D (400 IU/kg) diet (LD) or a diet containing whey protein, high calcium (1.5%) and vitamin D (10,000 IU/kg) diet (HD) and either high fat (40% of energy) or high sucrose (60%) base diets for 13 weeks. Liver tissue homogenates were used to determine [^{14}C] glucose and [^{14}C] palmitate oxidation. mRNA expression of enzymes related to energy metabolism in liver, adipose and muscle as well as regulators of muscle mass and insulin receptor were assessed. The results demonstrated that there was reduced accumulation of body fat mass ($P = 0.01$) and greater lean mass ($P = 0.03$) for the HD compared to LD fed group regardless of the background diet. There were no consistent differences between the LD and HD groups across background diets in substrate oxidation and mRNA expression for enzymes measured that regulate energy metabolism, myostatin or muscle VEGF. However, there was an increase in insulin receptor mRNA expression in muscle in the HD compared to the LD groups. In conclusion, elevated whey protein, calcium and vitamin D intake resulted in reduced accumulation of body fat mass and increased lean mass, with a commensurate increase in insulin receptor expression, regardless of the level of calories from fat or sucrose.

Keywords

Calcium; Vitamin D; whey; obesity; body composition; fat mass; lean mass; insulin; rats

1. Introduction

Obesity is a critical and growing problem worldwide. The estimates from the National Health and Nutrition Examination Survey (2003–2004) are that 32.9% of adults in the US are obese [1]. In addition, the prevalence of obesity continues to increase in adults, children and adolescents [1]. It is important to identify factors that may contribute to preventing the development of obesity in order to design strategies to reduce the impact of this health crisis.

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Epidemiologic and animal studies indicate an inverse relationship between dairy product intake and accumulation or loss of body fat mass [2]. Previous results from our laboratory demonstrate that calcium and dairy product intakes in humans during a 2 year interval were associated with reduced body fat mass accumulation [3]. Likewise, high calcium intake was associated with reduced fat mass accumulation in young (18–30 yr), normal weight women during an 18 month interval [4], though not at 12 months [5]. Evidence in rodent models demonstrated that elevated calcium or dairy product diets prevent fat mass accumulation [6;7]. However, not all studies, particularly intervention trials, support that calcium and dairy product have effects on body weight or fat mass accumulation [8]. Further, there is evidence that other dairy product components, whey protein [9] and vitamin D, may positively impact muscle mass or function. Thus, the impact of intakes of dairy products, or components such as calcium, on body composition remains controversial.

Several mechanisms have been proposed that may underlie the purported effects dairy product intake or components of dairy product intake on body composition [2]. First, increased fecal fat losses through the formation of calcium salts of fatty acids in the intestine and elevated fecal energy losses have been shown in several studies [7;10]. Second, results from our laboratory [10;11] and others [12;13], but not all [14], support that higher intakes of dairy product or calcium increases lipid oxidation. In addition, increased expression of uncoupling protein 2 (UCP2) in adipose tissue suggests an increased energy expenditure with elevated dairy product and calcium intake in a rodent model [15], though to our knowledge, no study supports that total energy expenditure is increased with high calcium or dairy intake [7;11; 14]. Further, the role of UCPs in thermogenesis in humans is controversial. In addition, the status of vitamin D, another component of dairy products, is associated with increased energy expenditure from a meal and increased lipid oxidation, independent of calcium or dairy product intakes, in overweight and obese participants in an intentional weight loss intervention [11]. Finally, whey protein is proposed to reduce myostatin, a negative regulator of muscle mass accumulation [16]. Overall, the impact of calcium, vitamin D and dairy products on body composition remains controversial, and suggests that other factors, other than calcium alone, may contribute to the putative impact of dairy products on body weight.

The mechanism by which dairy products regulate body composition remains controversial. The hypothesis of the current investigation is that elevated intakes of several components of dairy products (whey, calcium and vitamin D) reduces fat mass and increases lean mass accumulation, in the presence of either a high fat or a high sucrose background diet. To our knowledge, this is the first study to investigate the effects of dairy product component intake of a high sucrose background compared to the effect with a high fat background on regulation of body composition and the impact on important regulators of muscle mass.

2. METHODS and MATERIALS

2.1. Animals

Male Wistar rats (n=32, 175–190 gms) (Harlan Sprague Dawley, Indianapolis, IN) were individually housed in suspended wire cages and maintained on a 12 hour light/dark cycle at constant room temperature ($22 \pm 2^\circ\text{C}$). Animals were allowed to acclimate for one week.

2.2 Diets and Study Design

Animals randomly assigned to the experimental diets (Research Diets Inc., New Brunswick, New Jersey) shown in Table 1. The diets were based on the AIN 93G diet. In order to determine the interaction of macronutrient content of the diet with the whey, calcium and vitamin D contents of the diets, the diets contained either high fat with 40% of energy primarily from soybean oil or high sucrose with 66% of energy primarily from sucrose. Both high fat and high

sucrose diets contained low whey, low calcium and low vitamin D (LD) or high whey, high calcium and high vitamin D (HD). The LD and HD diets both contain casein, thus this study tested the effects of whey components with calcium and vitamin D, not the effects of dairy protein *per se*. An intake of 10,000 IU/kg diet of vitamin D was selected for these studies as the optimal level of vitamin D intake is currently under discussion in the scientific community [17;18] and this level of vitamin D does not lead to adverse effects [19]. Thus, the highest level of vitamin D that does not lead to adverse effects was selected to optimize the effects of vitamin D. Rats were fed the experimental diets for 13 weeks. Animals fed the HD diet showed no adverse effects in body weight, feed intake (Figure 1) or serum calcium (data not shown) for 13 weeks. The lack of adverse effects with high vitamin D and calcium intake results in the current study are similar to those of Fleet, et al. [19]. Food consumption was assessed from food and spillage weighed every two days. The protocol was approved by the Purdue Animal Care and Use Committee and complied with the Guide for the Care and Use of Laboratory Animals.

2.3 Body Weight and Body Composition

Body weights for all animals were measured weekly. During the 12th week of feeding the experimental diets, rats were anaesthetized using isoflurane and subjected to dual energy X-ray absorptiometry (DXA, Lunar Prodigy). Body fat mass and lean mass were determined using Lunar software version 4.3e (Lunar Corp., Madison, WI). The DXA was calibrated each day of analysis with a spine and standard calibration block, and the error was maintained within 1%.

2.4 Sample collection

At the end of the study, the rats were fasted overnight, euthanized with carbon dioxide overdose, decapitated and exsanguinated. Serum was stored at -80°C until analysis. At harvest an aliquot of liver tissue was transferred to ice- cold isolation buffer for substrate oxidation analysis (see below). Gastrocnemius muscle, liver and epididymal fat tissues were rapidly frozen in liquid nitrogen or in Trizol (Invitrogen, Carlsbad, CA) and stored in -80°C for use in estimating mRNA abundance.

2.5 Substrate Oxidation

Oxidation of glucose or palmitate in liver tissue was determined as previously described [20]. Briefly, liver tissue was homogenized immediately following harvest in four volumes of ice cold isolation buffer (mannitol 220 mM, sucrose 70 mM, HEPES 2 mM, EDTA 0.1 mM, pH 7.4) using a Potter-Elvehjem homogenizer and homogenates used to determine glucose and palmitate oxidation [19] with either 1.0 mM $[\text{U-}^{14}\text{C}]$ -palmitate or 5.0 mM $[\text{U-}^{14}\text{C}]$ -glucose. Palmitate was added in a 5:1 molar ratio complexed with BSA. Protein content of the homogenates was determined by the bicinchoninic acid protein assay (BCA, Pierce Biotechnology, Inc. IL). Substrate oxidation was calculated from specific activity and expressed as pmol substrate converted to product ($\text{mg protein}^{-1} \cdot \text{h}^{-1}$).

2.6 Biochemical Analysis of Serum

Nonesterified fatty acids and glucose were determined using quantitative colorimetric enzyme linked assays (NEFA-C kit and Autokit Glucose respectively; WAKO Chemicals, Richmond, VA). Serum levels of β -hydroxybutyrate were measured spectrophotometrically using the Liquicolor β -hydroxybutyrate kit (Stanbio Inc., Boerne, Texas).

2.7 Hepatic Acetyl Coenzyme A Carboxylase (ACC) Protein Levels

Hepatic activity of ACC was estimated by measuring phosphorylated/total ACC. Liver tissue was homogenized in isolation buffer (10 mM Tris-HCl, pH 7.5 containing 225 mM mannitol,

75 mM sucrose, 0.05 mM EDTA, 2.5 mM manganese chloride, 5 mM potassium citrate and 1 μ l/ml phosphatase inhibitor cocktail 2 (Sigma-Aldrich, St Louis, MO) and 1x Compleat (protease inhibitor cocktail; Roche, Switzerland)). Total protein was estimated in the supernatant fraction by BCA. Samples (20 μ g) were subjected to Western Blot analysis with 5% SDS-PAGE, and probed with antibodies for phospho-ACC (specific for the Ser79 residue of ACC), the blot stripped and reprobed for total ACC-2 (US Biological, Swampscott, MA). The proteins of interest were detected by ECL Advance reagents (Amersham Biosciences). Quantitative analysis was performed Fluor-STM MultiImager (Bio-Rad).

2.8 Gene Expression

Isolation of RNA from liver, muscle and adipose tissue was performed using TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total RNA was further purified using RNeasy and RNase-free DNase kits (Qiagen, Valencia, CA). cDNA was prepared using oligoDT and random hexamer primers and the Omniscript cDNA kit from Qiagen, according to the manufacturers protocol. Primers used are shown in Table 2. RT-PCR was carried out using Brilliant SYBR Green QPCR Master Mix (Stratagene, Cedar Creek, TX) in at least duplicate using a thermocycler (Mx3000P, Stratagene) as follows: 95°C for 30 seconds, 55°C for 1 minute, and 72°C for 30 to 40 seconds to achieve optimal amplification for each primer pair. Expression levels were determined by the Δ Ct method using GAPDH as control, except for muscle myostatin, VEGF, and insulin receptor expression for which 18S was used as control.

2.9 Statistics

Results were analyzed among all four dietary groups by two-way analysis of variance using SAS general linear model program (SAS/GLM Version 9.0, SAS Institute Inc., Cary, NC). Results of mRNA abundance are expressed as sample over mean of LD for each dietary background, and two tailed t tests were used to compare LD and HD groups from each dietary background (high fat or high sucrose). Differences were considered significant when $P < 0.05$.

3. RESULTS

Body weights did not differ for rats in the LD and HD groups fed either a high fat or high sucrose background (Figure 1A). There were no significant differences amongst groups in feed intake (data not shown) and no differences in calorie intake (Figure 1B) between the LD and HD groups within each base diet. Energy intake was greater in the high fat dietary groups compared to high sucrose dietary groups when main effect of base diet was assessed ($p=0.009$). Thus, though the high fat dietary group consumed more calories than the high sucrose dietary group, HD intake had no significant effects on body weight, calorie intake or feed intake compared to LD intake.

3.1 Body Composition

In order to determine the effects of dietary whey, vitamin D and calcium, body composition was assessed following 12 weeks of dietary intervention. Body fat mass (gm) was significantly lower in the groups consuming HD compared to LD with either a high fat or high sucrose dietary background (Figure 2A) with a main effect of LD compared to HD ($P < 0.01$). In addition, high dairy product component intake increased lean mass in the rats, with either the high fat or high sucrose dietary background (Figure 2B) with a main effect of LD compared to HD ($P < 0.04$). Thus, a shift in body composition from fat mass to lean mass occurred with HD intake with both dietary backgrounds.

3.2 Substrate Oxidation

To determine if substrate oxidation is altered in liver tissue between the LD and HD diets with either base diet, liver glucose and palmitate oxidation was assessed following dietary intervention. Liver glucose oxidation was elevated ($P < 0.05$) in the high fat LD group compared to the high fat HD group (Figure 3A). Liver palmitate oxidation was not different between high fat HD and high fat LD group ($P = 0.14$) (Figure 3B). Although, there was significantly greater palmitate oxidation in liver from rats fed a high fat diet compared with the high sucrose group ($p=0.0004$) when analyzed for main effect of dietary background, there were no significant differences between LD or HD treatments within the high sucrose diet group for glucose and palmitate oxidation in liver (Figure 3A and 3B). Thus, LD intake leads to an increase in glucose oxidation in liver with a high fat but not a sucrose diet.

3.3 Serum Biochemistry

There were no significant differences in serum glucose, nonesterified fatty acids and β -hydroxybutyrate between the LD and HD groups (Table 3). There was a main effect of dietary background with a significantly higher level of non-esterified fatty acids in the high sucrose dietary group compared to the high fat dietary group ($p=0.01$).

3.4 Liver Enzymes

The impact of the dietary intervention on mechanisms which regulate energy and substrate metabolism was assessed in liver tissue. There was no significant difference in the phosphorylated/total ACC protein expression, and therefore activity, in liver (Figure 4) between the LD and HD groups and no significant difference in main effect of dietary background. There were no significant differences in the liver in the mRNA expression for fatty acid synthase (FAS), phosphoenolpyruvate carboxykinase (PEPCK), carnitine palmitoyltransferase-1 α (CPT-1 α) and peroxisome proliferative activated receptor γ coactivator-1 α (PGC-1 α) (Table 4). To explore the possibility of increased energy expenditure in the liver and adipose tissue, mRNA expression of tissue specific UCPs was assessed. UCP2 (liver and adipose) and UCP3 (adipose) mRNA expression was not significantly different between the LD and HD groups with either base diet (Table 4).

3.5 Muscle Metabolism

Due to the increase in lean mass with the HD diet compared to the LD diet with both dietary backgrounds, muscle tissue was analyzed for mRNA expression of regulators of energy metabolism (ACC activity, CPT-1 β , PGC-1 α , UCP2 and UCP3) and potential hypertrophic regulators (vascular endothelial growth factor (VEGF), myostatin and insulin receptor). In muscle tissue, the expression of CPT-1 β was not different between the groups LD and HD groups with either dietary background (Table 5). There was no significant difference in ACC activity, assessed by phosphorylated ACC/total ACC protein (data not shown). There were no significant differences between LD and HD dietary groups in mRNA expression of VEGF or myostatin (Table 5). However, PGC-1 α was increased in muscle of animals in the HD compared with the LD dietary group with a high fat macronutrient background (Table 5), but not with sucrose dietary background. In contrast, the mRNA expression of the insulin receptor was significantly higher in the HD compared with the LD diet with both high fat and high sucrose dietary background (Table 5), with a significant main effect of LD compared to HD ($P < 0.02$).

4. Discussion

Our results demonstrate a reduced fat mass accumulation with intake of diets containing whey, high calcium and high vitamin D. The current study demonstrates for the first time that the

impact of these components in reducing fat mass accumulation is effective with either a high fat or high sucrose diet, and that the alterations in substrate oxidation in the liver differ with composition of base diet. Further, to our knowledge, this is the first study to demonstrate that high whey, calcium and vitamin D intakes increases insulin receptor expression in muscle coincident with an increase in lean mass regardless of base diet.

Our results showing reduced fat mass accumulation with whey, high calcium and high vitamin D diets compared to no whey, suboptimal dietary calcium and vitamin D is consistent with a variety of epidemiological trials in humans [2;3], and a follow-up of an intervention study [4]. In addition, the results of several calcium or dairy product intervention trials supports that calcium or dairy products enhance weight and fat mass loss in humans and animals [21;22]. However, not all weight loss trials support this conclusion [23;24]. In addition, several animal studies also support that dairy product intake reduces fat mass accumulation [6;7]. Therefore, the results of the current study confirm that the combination of several components of dairy products (whey, calcium and vitamin D) reduce fat mass accumulation in rodents.

HD diets reduced fat mass and increased lean mass regardless of whether the base diet contained high fat or high sucrose, suggesting that the primary mechanisms mediating these changes may be similar with both dietary backgrounds. The increased lean mass with high whey, calcium and vitamin D intake, with no differences in body weight, is the only consistent difference of the factors assessed across dietary backgrounds noted in this study that may influence energy utilization in tissues. Although the process of fat and lean mass accumulation may have similar energy costs, lean mass per kilogram is more metabolically active and requires greater energy utilization than fat mass. Thus, the increase in lean body mass may shift energy away from fat stores. The increase in insulin receptor expression in muscle is consistent with the increase in lean mass noted in this study. Insulin acts through the insulin receptor, a member of the receptor tyrosine kinase family and results from multiple studies suggest that insulin stimulates muscle cell hypertrophy [25,26]. Further, vitamin D status is associated with reduced risk of diabetes or higher insulin sensitivity [27], calcium intake is associated with reduced risk for metabolic syndrome [28], and whey protein may improve insulin sensitivity [29]. In addition, a vitamin D response element sequence has been found in the insulin receptor gene promoter [30]. Consistent with this, 1,25(OH)₂D increased the expression and transcription of the insulin receptor in pro-monocytic lymphoma cells [31]. The results of the current study are consistent with these data and suggest that dietary vitamin D, calcium and/or whey may enhance insulin action in muscle fibers through increased insulin receptor levels, potentially leading to increased lean mass. Therefore, it is hypothesized that dairy product components may improve lean mass, or contribute to maintenance of lean mass, without altering accumulation of body weight and thus it is critical to consider distribution of weight in the action of dairy products.

Other factors measured in this study that may impact muscle mass include myostatin and VEGF. Myostatin is a critical negative regulator of skeletal muscle cell hypertrophy, thus myostatin null mice (Mystn ^{-/-}) [32] have extraordinary amount of muscle primarily due to an abnormal increase in muscle fiber number, even though subtle increases in muscle fiber size have been noted. VEGF is important for the maintenance of the skeletal muscle capillaries [33,34]. Further, inhibition of VEGF production reduces skeletal muscle vascularization [35] and inhibition of VEGF during chronic electrical stimulation or exercise training inhibits skeletal muscle angiogenesis [33,36]. In the current study, there was no difference in either myostatin or VEGF mRNA expression between the LD and HD diets, suggesting that these factors may not play a role in the increase in lean mass with the HD diet.

The results of several studies suggest that a high calcium diet is associated with increased fat oxidation [10–13]. However, not all studies have shown an impact of higher calcium intake on fat oxidation [14]. It is intriguing that in the current study, high whey, calcium and vitamin D

intake modulates substrate utilization with a lower glucose oxidative capability with a high fat background, but not during high sucrose intake. Although tissue specific substrate oxidation may not reflect overall body oxidation, these results may contribute to understanding the discrepancy in the published results, as the macronutrient background is not considered in the studies to date. In addition, PGC-1 α expression was increased in the muscle in the HD compared to LD fed groups with a high fat diet. The transcriptional co-activator, PGC-1 α , is a key regulator of mitochondrial biogenesis. However, glucose oxidation and PGC-1 α were not altered in the liver of animals consuming the high sucrose diets and there were no differences in palmitate oxidation in either tissue between LD and HD containing diets. Cumulatively, the results of the current study suggest that the alterations in substrate oxidation mediated by higher whey, calcium and vitamin D intake noted during consumption of a high fat, but not a high sucrose background, are unlikely to contribute to changes in body composition because a lower body fat and higher lean mass accumulation was achieved with both dietary backgrounds.

In the current study, the increase in lean mass may play an important role in the coincident reduction in fat mass by shifting energy utilization. In several studies, dairy products enhance weight loss greater than calcium supplementation alone [21;22], suggesting another factor in addition to calcium in dairy products may impact body composition. Several factors in dairy products are proposed to contribute to energy balance or body composition, including protein or amino acid composition, calcium, or vitamin D. One possibility is the branched chain amino acid composition in milk, particularly leucine, is proposed to contribute to increased muscle mass [37]. In the current study the protein source was casein for all experimental diets, therefore the difference in amino acid composition between the diets was minimal. On the other hand, the HD diets contained whey proteins which are proposed to improve muscle mass, particularly under conditions of resistance training [38]. It is possible that the whey component of the high dairy product intake contributes to the increase in muscle mass. Alternatively, improved vitamin D status may also contribute to lean mass [39]. Support for this hypothesis is provided by studies in vitamin D receptor knock out mouse in which muscle fiber size is reduced [40]. Therefore, future studies are warranted to investigate which component, or combination of components, of the HD diet compared to the LD diet promoted an increase in lean mass and increases insulin receptor expression in muscle.

The strengths of the study are the comprehensive metabolic assessments, and body composition measurements. The limitations of the study are that the study focuses on metabolism in a rodent model, the use of growing animals and that the study applies to accumulation of body weight and may not apply to energy restricted regimens.

In summary, these results support that high whey, calcium and vitamin D intake prevents excess accumulation of fat mass with both high sucrose or high fat diets. Consistent results across macronutrient backgrounds suggest increased lean mass occurs with high whey, calcium and vitamin D intake compared to low intakes of these components. Finally, higher whey, calcium and vitamin D intake promotes increased expression of the insulin receptor in muscle in this rodent model, independent of dietary background. The specific component or components in the diets and the role of the insulin receptor in mediating the increase in lean mass warrants further investigation.

List of Abbreviations

LD, group of animals fed diets of suboptimal calcium (0.25%) and vitamin D (400 IU/kg); HD, group of animals fed whey protein, high calcium (1.5%) and vitamin D (10,000 IU/kg) diet; VEGF, Vascular endothelial growth factor; UCP, Uncoupling protein; DXA, Dual energy X-ray absorptiometry; ACC, Acetyl coenzyme A carboxylase; FAS, Fatty acid synthase;

PEPCK, phosphoenolpyruvate carboxykinase; CPT-1 α , carnitine palmitoyltransferase-1 α ; PGC-1 α , peroxisome proliferative activated receptor γ coactivator-1 α .

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Figure 1. Body weight and Calorie Intake

Body weights during dietary 13 week intervention with LD or HD diets with either a high fat or high sucrose base are expressed as mean $\pm$ SE. There were no significant differences between the groups as evaluated by two-way analysis of variance. Calorie intake (B) values are expressed as means $\pm$ SE and the number of animals in each group is shown in the bar. Bars with different letters are significantly different as assessed by two-way analysis of variance.

Figure 2. Fat Mass and Lean Mass

Following 13 week dietary intervention with LD or HD diets with either a high fat or high sucrose base, fat mass (A) and lean mass (B) of animals was estimated by DXA. Values are expressed as mean $\pm$ SEM. Bars with different letters are significantly different as assessed by two-way analysis of variance (ANOVA).

Figure 3. Liver Substrate Oxidation

Following 13 week dietary intervention with LD or HD diets with either a high fat or high sucrose base, liver oxidation of glucose (A) and palmitate (B) was assessed. Values are expressed as mean $\pm$ SE (pmols CO₂/mg protein/hour). Bars with different letters are significantly different as assessed by two-way analysis of variance.

Figure 4. Liver ACC Activity

Following 13 week dietary intervention with LD or HD diets with either a high fat or high sucrose base animals were harvested. Western blot analyses was completed to assess hepatic activity of ACC employing total and phospho-specific antibodies and the quantification of the Western for phosphorylated/total ACC in the intervention groups are shown. Values are expressed as mean±SEM. There was no significant difference amongst the groups or between LD and HD groups when results were collapsed by main effect of base diet in GLM model.

Table 1

Content of Intervention Diets*

	High Fat		High Sucrose	
	LD	HD	LD	HD
Casein (gm)*	200	149	200	149
Non-Fat Dry Milk (gm)	0	125	0	125
Protein (% of energy)	18	18	18	18
Corn Starch (gm)	166	99	67	0
Maltodextrin	132	132	0	0
Sucrose	100	100	562	562
Cellulose	50	50	50	50
Carbohydrate (% of energy)	42	42	66	65
Soybean Oil	173	173	70	70
Fat (% of energy)	40	40	16	16
Calcium (%)	0.25	1.5	0.25	1.5
Phosphate (%)	0.25	0.75	0.25	0.75
Vitamin D ₃ (IU/kg)	400	10000	400	10000

* All diets contain adequate vitamins and minerals unless indicated otherwise. Equivalent t-Butylhydroquinone (0.014 gm/3904 calories) and 3 gm added L-cystine to each diet.

Table 2

Primers

Protein	Forward Primer	Reverse Primer
Fatty Acid Synthase	TGC AGA GCA TAG CAA GGT GCT AGA	ACA GGT TGT AAT CGG CAC CCA AGT
Carnitine palmitoyl transferase-1α (CPT-1α)	TTG GGA AGC ACT TGA GAC AAC CCA	TGT GCC CAA TAT TCC TGG AGC CAA
CPT-1β	TGA AGT CAT GGT GGG CAA CCA ACT	AGC CAA ACC TTG AAG AAG CGA CCT
Phosphoenolpyruvate carboxykinase	ACC CAC AGG ATC AGA ACA AAC CCT	TCC GAA CGC CAT TAA GAC CAT CCA
Peroxisome proliferator activated receptor gamma coactivator-1α (PGC-1α)	ACC CAC AGG ATC AGA ACA AAC CCT	ACT GCG GTT GTG TAT GGG ACT TCT
GAPDH	AAC GGC ACA GTC AAG GCT GAG AAT	TCT CGT GGT TCA CAC CCA TCA CAA
18S	TTA GAG TGT TCA AAG CAG GCC	TCT TGG CAA ATG CTT TCG CTC TGG
Uncoupling Protein 2 (UCP2)	AAG CAG TTC TAC ACC AAG GGC TCA	AAT GGC ATT TCG AAC ATT GGG
Uncoupling Protein 3 (UCP3)	GAA GCT GCT GGA TC TCA CC	CGT TTC AGC TGC TCG TAG GT
Vascular Endothelial Growth Factor (VEGF)	AGG AAA GGG AAA GGG TCA AA	AAT GCT TTC TCC GCT CTG AA
Myostatin	ACG CTA CCA CGG AAA CAA TC	CCT GGG CTC ATG TCA AGT TT
Insulin Receptor	ATC CCG AAA GCG AAG ATC CCT TGA	GGT TCC TTT GGC TCT TGC CAC ATT

Table 3

Serum Levels of Non-esterified fatty acids, β Hydroxybutyrate and Glucose Following Dietary Interventions (Mean $\pm$ SE)

	High Fat		High Sucrose	
	LD	HD	LD	HD
Non-esterified fatty acids (mmol/L)	0.92 $\pm$ 0.11	0.89 $\pm$ 0.07	1.10 $\pm$ 0.02	1.10 $\pm$ 0.09
β Hydroxybutyrate (mmol/L)	0.92 $\pm$ 0.24	0.78 $\pm$ 0.2	0.99 $\pm$ 0.26	0.92 $\pm$ 0.23
Glucose (mmol/L)	150 $\pm$ 31	158 $\pm$ 23	140 $\pm$ 11	137 $\pm$ 7

The diets were based on the AIN 93G diet with either high fat with 40% of energy primarily from soybean oil or high sucrose with 66% of energy primarily from sucrose. Low fat and high fat diets contained low whey, low calcium and low vitamin D (LD) or high whey, high calcium and high vitamin D (HD). There were no significant differences among the groups for any parameter.

Table 4

mRNA Expression of Enzymes in Liver and Adipose Tissue Following Dietary Interventions (Means±SE)

	High Fat		High Sucrose	
	LD	HD	LD	HD
Liver CPT-1^{ab}	1.00±0.24	1.51±0.24	1.00±0.21	1.19±0.35
Liver FAS^c	1.00±0.32	0.82±0.09	1.00±0.10	1.46±0.21
Liver PEPCK^d	1.00±0.36	0.53±0.11	1.00±0.35	1.44±0.38
Liver PGC-1α^e	1.00±0.23	1.44±0.40	1.00±0.10	1.08±0.09
Liver UCP2^f	1.00±0.12	0.79±0.12	1.00±0.09	0.89±0.13
Adipose UCP2	1.00±0.19	1.00±0.14	1.00±0.20	0.91±0.16
Adipose UCP3	1.00±0.15	0.82±0.15	1.00±0.15	1.20±0.19

The diets were based on the AIN 93G diet with either high fat with 40% of energy primarily from soybean oil or high sucrose with 66% of energy primarily from sucrose. Low fat and high fat diets contained low whey, low calcium and low vitamin D (LD) or high whey, high calcium and high vitamin D (HD).

Abbreviations are carnitine palmitoyltransferase-1 (CPT-1), fatty acid synthase (FAS), phosphoenolpyruvate carboxykinase (PEPCK), peroxisome proliferative activated receptor γ coactivator-1 α (PGC-1 α), uncoupling protein (UCP). Data is expressed over LD average for background diet. Statistical analyses compared LD and HD within dietary background (high fat or high sucrose) by two tailed T test.

Table 5

mRNA Expression of Enzymes in Muscle Tissue Following Dietary Interventions (Means±SE)

	High Fat		High Sucrose	
	LD	HD	LD	HD
CPT-1β^a	1.00±0.23	1.45±0.32	1.00±0.14	0.99±0.17
PGC-1α^b	1.00±0.13	2.32±0.24 [*]	1.00±0.23	0.71±0.06
UCP2^d	1.00±0.24	0.65±0.04	1.00±0.13	1.07±0.18
UCP3	1.00±0.14	0.78±0.18	1.00±0.30	0.92±0.18
VEGF^c	1.00±0.19	0.86±0.19	1.00±0.25	1.28±0.24
Myostatin	1.00±0.20	1.14±0.30	1.00±0.34	0.95±0.29
Insulin Receptor	1.00±.18	2.20±0.45 [*]	1.00±0.40	1.94±0.39 [*]

^a Data is expressed over LD average for background diet. Statistical analyses compared LD and HD within dietary background (high fat or high sucrose) by two tailed T test.

Asterisk (*) indicates significant difference between HD and LD groups within dietary background.

Abbreviations are carnitine palmitoyltransferase-1 β (CPT-1 β), Peroxisome proliferative activated receptor γ coactivator-1 α (CPT-1 α), Uncoupling Protein (UCP) and Vascular Endothelial Growth Factor (VEGF).