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Cessation of daily wheel running differentially alters fat oxidation capacity in liver, muscle, and adipose tissue

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Running Head:
Lipid oxidation in the transition to physical inactivity

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Abstract

Physical inactivity is associated with the increased risk of developing chronic metabolic diseases. To understand early alterations caused by physical inactivity, we utilize an animal model in which rats are transitioned from daily voluntary wheel running to a sedentary condition. In the hours and days following this transition, adipose tissue mass rapidly increases, due in part to increased lipogenesis. However, whether a concurrent decrease in fatty acid oxidative capacity (FAO) in skeletal muscle, liver, and adipose tissue occurs during this period is unknown. Following six weeks of access to voluntary running wheels (average distance of ~6 km a night), rats were rapidly transitioned to a sedentary state by locking the wheels for 5 hours (WL5) or 173 hours (WL173). Complete (^{14}C palmitate oxidation to $^{14}CO^2$) and incomplete (^{14}C palmitate oxidation to ^{14}C labeled acid soluble metabolites) was determined in isolated mitochondrial and whole homogenate preparations from skeletal muscle and liver and in isolated adipocytes. Strikingly, the elevated complete FAO in the red gastrocnemius at WL5 fell to that of rats that never ran (SED) by WL173. In contrast, hepatic FAO was elevated at WL173 above both WL5 and SED groups; while in isolated adipocytes, FAO remained higher in both running groups (WL5 and WL173) compared to the SED group. The alterations in muscle and liver fat oxidation were associated with changes in carnitine palmitoyl transferase-1 activity and inhibition, but not significant changes in other mitochondrial enzyme activities. In addition, PGC-1α mRNA levels that were higher in both skeletal muscle and liver at WL5 fell to SED levels at WL173. This study is the first to demonstrate that the transition from high to low daily physical activity causes rapid, tissue-specific changes in FAO.

Introduction

Alterations in our current environment, including physical inactivity and the excessive consumption of high calorie/fat foods, are root causes for the rapid rise of obesity and diabetes in humans (1;6;15;41). Although many investigations have detailed the role of over-nutrition following changes in substrate utilization and storage, the effects of a transition from high to low physical activity remain largely understudied and underappreciated.

Our research group utilizes "the rodent wheel lock model" to study acute transitions from high to low levels of daily physical activity (26;27;30;34;38). After days or weeks of daily voluntary wheel running (5-15 km/night), wheels are locked for progressive periods of time (from 5 to 173 hours) providing an acute window of time to study the metabolic alterations that ensue. While resulting in only modest increases in mitochondrial enzymes compared to forced treadmill training (11;28), voluntary wheel running (5-15 km/night) can increase energy expenditure far greater than treadmill training (~2 km/60 min treadmill bout). Thus, it is a good model to study the transition from high to low daily physical activity, but an inappropriate model to study detraining. In our previous studies, the sudden cessation of daily running caused rapid and robust increases in the weight of retroperitoneal and omental fat pads after 53 and 173 hours of wheel lock whose causes are beginning to be explained by intrinsic changes in substrate partitioning and not simply a positive energy balance due to decreased daily energy

expenditure (26;30). For example, rates of key enzymes in adipose tissue *de novo* lipogenesis and palmitate incorporation into adipose triglycerides remain significantly higher in the hours and days immediately following wheel lock (26;27) and after the cessation of forced treadmill running (9). Although, enhanced *de novo* lipogenesis could partially explain the rapid increases in fat pad weight after the cessation of activity, we also speculated that rapid decreases in fatty acid oxidative capacity (FAO) of skeletal muscle, liver, and adipose tissue also may play a significant role.

Decreased FAO in metabolic tissues has been strongly associated with chronic disease(s) (5;13;14;16;20;21;42). Though findings are not universal (12;25), extremely obese and type 2 diabetic subjects display reduced systemic and skeletal muscle FAO, which has been linked to increased adiposity, increased intramuscular lipid storage, and skeletal muscle insulin resistance (5;14;21;44). Decreased hepatic FAO directly causes or increases susceptibility for fatty liver and steatosis (17;39) and also has been linked to increased susceptibility to diet-induced obesity (18). In addition to skeletal muscle and liver FAO in adipocytes has become an important area of study. FAO is decreased in the adipose tissue of *db/db* mice (7) and mitochondrial markers are also reduced in the adipocytes of several animal models of type 2 diabetes and obesity (8;13;16;20;42;46). However, while it is well known that increased physical activity decreases the risks of type 2 diabetes and obesity it remains unknown whether physical activity modifies adipocyte FAO.

The aim of this study was to determine if the cessation of voluntary wheel running leads to concurrent alterations in tissue fatty acid catabolism, carnitine palmitoyl transferase-1 (CPT-1) activity and regulation, and mitochondrial enzyme activity. Since

the transition from high to low physical activity is likely to cause systemic changes, we assessed FAO and mitochondrial function in adipose tissue, liver, and mixed skeletal muscle in hopes of better understanding the integrative response(s) of an organism to reductions in energy expenditure and rapid gains in adiposity. We hypothesized, that the transition from high to low physical activity would lead to a coincident reduction in FAO and mitochondrial enzyme activity in skeletal muscle, liver, and adipocytes.

Methods

Animal protocol. The animal protocol was approved by the Institutional Animal Care and Use Committee at the University of Missouri-Columbia. Thirty-six, male Fischer 344 x Brown Norway F1 hybrid rats (Harlan) were obtained at 21 days of age. Animals were randomly separated into those with access to running wheels (WL) and those without access to the wheels (Sed). Rats assigned to running groups were immediately housed (at the age of 21 days) in cages equipped with a voluntary running wheels outfitted with a Sigma Sport BC 800 bicycle computer (Cherry Creek Cyclery, Foster Falls, VA) for measuring daily running activity. Voluntary running was selected to approximate the more natural physical activity level of the animal. Rats were maintained on a 12-h light, 12-h dark cycle that ran from 0600 to 1800. Running activity was obtained every day of running between 0800 and 1000, and rats in the running group had access to wheels and food and water ad libitum until ~9 wk of age, at which time (0600) wheels were locked. Rats were anesthetized [ketamine (80 $mg \cdot kg^{-1}$), xylazine (10 $mg \cdot kg^{-1}$), and acepromazine (4 $mg \cdot kg^{-1}$)] and killed by exsanguination by removal of the heart either 5 h (WL5), or 173 h (WL173) after locking of wheels; the sedentary rats (SED) were sacrificed at the same time. All animals were fasted for 5 hours prior to sacrifice.

Dual-energy X-ray absorptiometry (DEXA). One week prior to and the day of sacrifice animals were anesthetized with isoflurane (one week prior) or ketamine (80 $mg \cdot kg^{-1}$), xylazine (10 $mg \cdot kg^{-1}$), and acepromazine (4 $mg \cdot kg^{-1}$) (day of sacrifice) and whole-body composition was measured using a Hologic QDR-1000/w DEXA machine calibrated for rats.

Serum measures. Plasma glucose (Sigma), triglycerides (Sigma), and free fatty acids (FFAs; Wako Chemicals, Richmond, VA) were measured with commercially available kits according to the manufacturer's instructions.

Adipocyte isolation. Adipocytes from epididymal fat pads were isolated by a modification of the Rodbell method (26;40). Fresh triplicate 30 μL aliquots of packed adipocytes were used for FAO and a single 30 μL aliquot frozen at -80° C for DNA content, with the remainder lysed in Cell Lytic (Sigma, USA) for enzyme activity assays.

Adipocyte DNA Content. DNA content in isolated adipocytes was determined by the flurometric assay described by Labarca and Paigen (29). Briefly, packed adipocytes were lysed in 2M NaCl, 50 mM $NaPO_4$ (pH 7.4) and 20 ul added to 80 μL of working solution (0.04 μg/mL of Hoeschst #33258, 10 mM Tris-HCl, 1mM EDTA, 200 mM NaCl, pH 7.4) in a 96-well plate. Samples were excited at 356 nm resulting in an emission at 458 nm. Calf thymus DNA (Sigma, USA) was used to generate a standard curve.

Tissue homogenization procedure. Red gastrocnemius and livers were quickly excised from anesthetized rats and placed in ice-cold isolation buffer (100 mM KCl, 40 mM Tris·HCl, 10 mM Tris-base, 5 mM $MgCl_2 \cdot 6H_2O$, 1 mM EDTA, and 1 mM ATP; pH 7.4). For fresh acid oxidation assays, ~50–100 mg of tissue was thoroughly minced with scissors in 200 μl SET buffer (250 mM sucrose, 1 mM EDTA, 10 mM Tris·HCl, and 2 mM ATP; pH 7.4) and then the buffer volume was brought up to yield a 20-fold (wt/vol) diluted sample. This was transferred to a 3-ml Potter-Elvehjem glass homogenization vessel. Tissue suspensions were mechanically homogenized on ice with a Teflon pestle at

10 passes over the course of 30 s at 1,200 rpm. Homogenates were kept on ice until oxidation experiments were performed.

Mitochondrial isolation from red gastrocnemius skeletal muscle and liver. Mitochondrial suspensions were prepared according to modified methods of Koves et al. (23;24). A portion of the red gastrocnemius and liver was quickly excised from unconscious rats and placed in 5 ml of fresh *buffer A* (100 mM KCl, 50 mM MOPS, 5 mM MgSO4, 1 mM EGTA, 1 mM ATP, pH 7.4). The tissue was then minced and suspended 7-fold (wt/vol) in *buffer A*, and homogenized for 15 s. The homogenate was then centrifuged at 800 *g* for 10 min at 4°C and the supernatant was filtered through gauze and set aside. The remaining pellet was resuspended in *buffer A*, homogenized, and the homogenate was then centrifuged at 800 g for 10 min at 4°C. The remaining supernatant was added to the previously set aside supernatant after filtering through gauze. The pooled supernatants were centrifuged at 9,000 *g* to pellet the mitochondria. The pellet was resuspended in *buffer B* (*buffer A*, 0.2% BSA, pH 7.4), and centrifuged at 8,000 *g* before the pellet was washed and resuspended in *buffer C* (100 mM KCl, 50 mM MOPS, 0.5 mM EGTA, pH 7.4) and centrifuged for 10 min at 7,000 *g*. The final pellet was resuspended in 0.5 ml of sucrose EDTA (SET) buffer and protein content was determined.

Fatty acid oxidation. FAO was measured with radiolabeled [1-^{14}C]palmitate (American Radiochemicals) in fresh red gastrocnemius skeletal muscle and liver homogenates and isolated mitochondria using modified methods of Dohm et al.(10;39). The reaction buffer used for whole homogenates utilized a cold palmitate concentration of 200 μM while the reaction buffer used for isolated mitochondria utilized a

concentration of 50 μM. For isolated adipocytes, 30 μl of freshly isolated adipocytes/reaction was added to 120 μl reaction buffer in a trapping device with an adjacent chamber containing 100 μl of NaOH that captured the $^{14}CO_2$, representing the complete oxidation radiolabeled palmitate, when 100 μl of perchloric acid was added to the reaction following a two-hour incubation at 37º C. The ^{14}C labeled acid soluble metabolites, representing incomplete FAO were also collected.

Citrate synthase, beta-hydroxyacyl-CoA dehydrogenase (β-HAD), and cytochrome c oxidase activity. Citrate synthase and β-HAD activities were determined in red gastrocnemius, liver, and adipocyte homogenates using the methods of Srere et al (43) and Bass et al (4), respectively, and as previously described by our group (39). β-HAD activity was undetectable in the adipocyte fraction. Cytochrome c oxidase activity was determined by a commercially available kit (Sigma, St. Louis, MO, USA) in all three tissues as previously described by our group (38). All assays were performed at 37˚C.

CPT-1 assay CPT-1 activity and inhibition by malonyl-CoA using modified methods of Koves et al. (25). 90 μl of reaction buffer (117 mM Tris HCl, 0.28 mM reduced Glutathione, 4,4 mM ATP, 50 uM Palmitoyl-CoA, 4 mg/ml Rotenone, 0.1% BSA) containing 0.2 mM [^{3}H]-Carnitine and either 0 or 10 μM malonyl-CoA was added to 10 μl of isolated mitochondria of red gastrocnemius skeletal muscle and liver homogenate for 6 min at 37^{o}C. [^{3}H]palmitoyl-carnitine formed was extracted with 350 μl of water-saturated butanol, quantified by liquid-scintillation counting, and normalized to protein content.

Statistics. Each outcome measure was examined in 6-8 animals. For each outcome measure, a one-way analysis of variance was performed (Sigma Stat). However, if

normality of the data set failed, a Kruskal-Wallis ANOVA on ranks was performed, with Dunn's method comparison (control group = WL5) as the post-hoc test. A significant main effect ($p<0.05$) was followed-up with Student-Newman-Kuel post-hoc comparisons. Values are reported as means ± standard error of the mean (SE), and a $P < 0.05$ denotes a statistically significant difference.

Results

Body Composition. DEXA measures were taken 7 days prior to, and on the day of sacrifice to capture any changes leading to sacrifice. In the 173-hour wheel lock period, the WL173 group increased percent body fat and total body fat mass by 45% and 46%, respectively, ($p < 0.05$), essentially returning body fat levels to those measured in the SED group (Table 1). No changes in percent or total body fat mass occurred in the 7 days prior to sacrifice in the WL5 or SED groups. Intriguingly total lean mass showed a different pattern than total fat mass (Table 1). Total lean mass increased in WL5 and SED in the 7 days prior to sacrifice suggesting a positive protein balance ($p < 0.05$). In contrast the WL173 group did not show a net increase in lean body mass during the 7 days of wheel lock prior to sacrifice, suggesting different rates of lean mass catabolism and/or anabolism during the 7 days following the cessation of running. All groups gained equal body mass in the 7 days prior to sacrifice.

Food Intake and Running Distances. Average daily food intakes during the last week of life for WL5 and WL173 were 27% and 37%, respectively, higher than SED ($p<0.05$) (Table 1). This is consistent with our previous findings that voluntary wheel running rats have greater food intakes than sedentary counterparts in this strain, age and gender of rat (26;30). During the 7 days of wheel lock, the WL173 group decreased their average daily food intake by 12% ($p<0.05$) as compared to the previous week; at the same time, food intake by the SED group increased 11% ($p<0.05$). Therefore, any differences detected were not due to overeating by the WL173 group since they ate a similar level to the SED during the 173 hrs of WL.

Neither total 6-wk nor average daily running distances (6.12 vs. 5.43 km/day, p=0.42) differed between WL5 and WL173 groups. The average daily running distance in the last week of running was 8.87 km and 9.73 km in WL5 and WL173 groups, respectively (p=0.68).

Fat Pad and Serum Characteristics. Absolute epididymal fat masses did not differ among groups (p=0.086), but when normalized to total mass, WL5 were lower than WL173 by 25% ($p<0.05$). Serum triglycerides (p=0.146), FFAs (p=0.188), and glucose (p=0.147) did not statistically differ among WL5, WL173, and SED groups (Table 2).

Fatty Acid Oxidation and Mitochondrial Enzyme Activities. Complete [$^{14}CO_2$] and incomplete [^{14}C labeled acid soluble metabolites (ASM)] capacity for palmitate oxidation was determined in isolated mitochondria or whole tissue homogenates from red gastrocnemius muscle and liver. In addition, complete and incomplete FAO was also measured in isolated adipocytes from epididymal fat pads. For whole red gastrocnemius homogenate, palmitate oxidation at WL5 was 33% and 50% higher than WL173 and SED, respectively, (p=0.059; Figure 1A). For isolated mitochondria from the red gastrocnemius muscle, complete palmitate oxidation to CO_2 was 91% higher at WL5 compared with SED ($p<0.05$, Figure 1B) but did not differ from WL173 (p=0.24).

In a contrast to what was found in the red gastrocnemius skeletal muscle, complete hepatic FAO increased after the transition to physical inactivity. Whole liver homogenates from the WL173 group completely oxidized 65% and 46% more palmitate than WL5 and SED groups respectively ($p<0.05$; Figure 2A). Total palmitate oxidation (CO_2 + ASMs) in liver did not differ among groups, an effect potentially in part due to the contribution of extra-mitochondrial peroxisomal activity. In addition, complete

oxidation in the isolated liver mitochondria was not statistically different among groups, but showed a similar trend as the whole homogenate (Figure 2B).

In isolated epididymal adipocytes, complete palmitate oxidation was 81% higher in the WL5 compared to SED ($p<0.05$) and 71% higher in the WL173 compared to SED ($p<0.05$), but not different between WL5 and WL173 when normalized to the volume of packed adipocytes (Figure 3A). However, when complete palmitate oxidation was normalized to DNA content (ng of DNA/30μL of packed adipocytes) WL173 no longer remained significantly higher than SED group (Figure 3B). Total palmitate oxidation (CO_2 + ASMs) in the adipocytes did not differ among groups.

No statistically significant differences in β-hydroxy-acetyl-CoA dehydrogenase, citrate synthase, or cytochrome c oxidase activities in red gastrocnemius, liver, or isolated adipocytes were detected among groups (Table 3).

CPT-1 activity. In isolated mitochondria from red gastrocnemius muscle, the WL5 group had 41% higher baseline CPT-1 activity than the WL173 group ($p<0.05$) but WL5 did not differ from SED. As expected, the addition of 10 μM malonyl-CoA caused decreases in CPT-1 activity in all three groups (Figure 4). Unexpectedly, the change in CPT-1 activity from baseline to 10 μM malonyl-CoA was nearly twice as much (delta) in the WL5 group compared to the WL173 and SED groups ($p<0.05$), suggesting a greater CPT-1 sensitivity to malonyl-CoA in a physically active condition (WL5) that was lost after 7 days of lowered physical activity (WL173). No differences in the CPT-1 activity of isolated mitochondria from liver existed among groups for 0 or 10 μM malonyl-CoA concentrations.

PGC-1α mRNA Levels. PGC-1α mRNA levels were significantly higher at WL5 compared with the SED group in both red gastrocnemius and liver (Figure 5A, 5B). The transition from WL5 to WL173 led to a 35% and 68% decrease in red gastrocnemius ($p = 0.05$) and liver ($p < 0.05$) PGC-1α mRNA levels, respectively. Consequently, WL173 and SED groups PGC-1α mRNA did not differ in red gastrocnemius (Figure 5A). Remarkably in the liver, WL173 PGC-1α mRNA fell to levels significantly less than the SED group (Figure 5B). In epididymal adipose tissue, PGC-1α mRNA did not differ among groups (Figure 5C).

Discussion

The transition from high to low physical activity in our rat model caused: 1) dissimilar responses for FAO in skeletal muscle (decreasing from its elevated value in daily running), liver (increasing from its unchanged value in daily running), and adipose tissue (maintenance of its increased value in daily running); 2) decreased CPT-1 activity and increased CPT-1 sensitivity to malonyl-CoA inhibition in muscle; 3) reduced PGC-1α mRNA in both skeletal muscle and liver; and 4) impaired growth of lean body mass compared to animals that sustain wheel running or the sedentary condition.

The transition from high to low physical activity likely decreases FAO in skeletal muscle by decreasing cellular energy requirements and decreasing PGC-1α expression, a critical transcriptional co-activator that coordinates increases in FAO enzyme, mitochondrial biogenesis, and a general increase in oxidative capacity. Observing similar trends in both homogenate and mitochondrial fraction leads us to speculate that the changes in FAO are not entirely due to a mass action effect, but also in part to an inherent change in mitochondrial "quality", which may be related to PGC-1α expression.

PGC-1α, as a co-activator, putatively increases FAO by increasing the promoter activity and thus expression of pyruvate dehydrogenase kinase-4 and CPT-1 (31;47). This results in more acetyl CoA being derived from fatty acids and increased fatty acid entry into the mitochondria (23). Unlike muscle, where energy derived from FAO fuels muscle contraction, an increase in hepatic PGC-1α mediated FAO provides a key source of ATP for ketogenesis and gluconeogenesis during fasting conditions (48). A limitation of the current study is that we did not measure oxidation of substrates, malate or pyruvate, that are commonly used to assess mitochondrial function.

Exercise causes an acute and transient increase in skeletal muscle PGC-1α gene expression (2;37). Conversely, we show here that the cessation of daily physical activity reduces skeletal muscle PGC-1α expression in 173 h supporting earlier work in which more extreme forms of physical inactivity reduced skeletal muscle PGC-1α in humans (45). We also witnessed that CPT-1 activity, a downstream target of PGC-1α, decreased in red gastrocnemius skeletal muscle after the cessation of daily running. Reduced PGC-1α expression in skeletal muscle has been linked to insulin resistance in sedentary subjects (33;36), leading us to speculate that a physical inactivity induced drop in PGC-1α is linked to skeletal muscle insulin resistance.

To our knowledge, this is the first report to measure changes in hepatic PGC-1α expression after chronic voluntary exercise or after a transition to inactivity. We speculate that daily exercise increases hepatic PGC-1α in a similar manner to fasting as both stimuli rely on increased hepatic glucose output to restore or sustain plasma glucose homeostasis. Interestingly, in contrast to what was found in muscle, the reduction in hepatic PGC-1α expression found after wheel lock was associated with increased complete hepatic FAO and no change in CPT-1 enzyme activity. Further research is needed to fully understand hepatic PGC-1α gene expression, its regulation by exercise, and its role in controlling hepatic FAO.

Intracellular accumulation of malonyl-CoA reduces fat and increases glucose utilization by the mitochondria (3;32). The current findings show a greater reduction in CPT-1 activity in the presence of 10 uM malonyl-CoA in the WL5 group suggesting that skeletal muscle mitochondrial fatty acid entry may be more tightly regulated in wheel running rats, than SED rats. Potentially, this would allow for skeletal muscle to be more

responsive in switching substrate utilization to match current energy demands. In addition, the CPT-1 responses to 10 uM malonyl CoA were similar between SED and WL173 skeletal muscle, suggesting that the enhanced exercise-induced regulation of CPT-1 by malonyl CoA is quickly lost if daily physical activity is not maintained. A lack of malonyl CoA inhibition is also noted with type 2 diabetes, where isolated skeletal muscle mitochondria have greater FAO than healthy controls despite having elevated malonyl CoA levels (3). Collectively, these observations suggest that physical activity modulates plasticity for malonyl-CoA's effects on skeletal muscle CPT-1 activity.

We speculate that the observed increase in complete FAO in liver during 7-day periods of decreased physical activity, in addition to the expansion of adipose tissue, may help prevent excess lipid storage in lean animals on a low-fat diet. The findings that complete hepatic FAO is increased after the cessation of wheel running is opposite to our previous findings in hyperphagic, obese OLETF rats in which the cessation of wheel running for 7 days reduced hepatic palmitate oxidation to sedentary levels (38). These differences may be due to the larger body fat stores and /or older age in the OLETF rat. It is important to emphasize that the witnessed changes were for complete FAO (^{14}C palmitate to $^{14}CO_2$) or the complete breakdown of fatty acids through the TCA cycle, rather than the incomplete oxidation of fatty acids (^{14}C palmitate to ^{14}C labeled acid soluble metabolites) in which fatty acids are only processed through β-oxidation. The difference between complete and incomplete FAO is significant for two reasons, 1) only complete FAO is coupled to significant ATP production, and 2) as suggested by Koves et al. complete FAO is less likely to produce intermediate metabolites that may negatively impact metabolic processes (25). In addition, our FAO measurements represent the

maximal capacity or potential to oxidize fatty acids, and because they are not in-vivo measures they should be interpreted cautiously.

Unlike skeletal muscle and liver, adipocyte FAO remained elevated over the SED group with 7 days of physical inactivity. Mitochondrial DNA number (mtDNA) from human subcutaneous fat is positively associated with increased lipogenic enzymes (19), and we previously have demonstrated that cessation of voluntary wheel running increased protein and enzyme activity for mitochondrial glycerol-3-phosphate acyltransferase, a rate-limiting lipogenic enzyme, in adipose tissue in rats (27). In addition, although our unpublished preliminary work has found no significant change in red gastrocnemius or liver triglyceride content, there is a significant increase in adipocyte triglyceride deposition after the cessation of wheel running in wild-type rats on a low-fat diet (26;30). The exercise-induced increase in adipose FAO in adipose tissue with ceased running may partially supply ATP for free fatty acid reesterfication, an energy costly process, or the increased ATP may be used to synthesize adipokines like adiponectin, a process shown to require functional mitochondria (22).

To our knowledge this is the first evidence that the cessation of voluntary running acutely (for at least 173 h) attenuates lean body mass growth. We have also recently reported that lean body mass significantly decreases in young healthy men who decrease their daily walking from 10,501 to 1,344 steps for 2 wks (35). The apparent change in lean mass in both studies leads us to question if the attenuated growth or loss of muscle mass in both studies is linked to a repartitioning of glucose and amino acids away from skeletal muscle (loss of insulin sensitivity) and/or decreased FAO. Because of the

importance of lean body mass in metabolic health, future research is needed to study these interactions.

In conclusion, we show that FAO in skeletal muscle, liver, and adipocytes display strikingly different responses to a 1-wk transition from high to low daily physical activity, a period in which fat pad mass increases rapidly. In addition, we found that two factors associated with metabolic disease, skeletal muscle FAO and PGC-1 α, were both reduced after the cessation of daily activity. These results support our overall hypothesis that the cessation of daily physical activity quickly alters metabolic function in a manner that likely precedes the later development of chronic disease should physical inactivity continue.

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Figure Legends

Figure 1 – Complete oxidation of palmitate to CO_2 in whole red gastrocnemius homogenate (Panel A) and isolated mitochondria from red gastrocnemius (Panel B). In Panel A there is a main effect p-value of 0.059. Values (means± SE; n=6-8/group) with different letters are significantly different ($p<0.05$).

Figure 2 - Complete oxidation of palmitate to CO_2 in whole liver homogenate (Panel A) and isolated mitochondria from liver (Panel B). Values (means± SE; n=6-8/group) with different letters are significantly different ($p<0.05$).

Figure 3 - Complete oxidation of palmitate to CO_2 in isolated adipocytes from the epididymal fat pad normalized to ng of DNA per 30µL of packed adipocytes (Panel A) or to volume (µL) of packed adipocytes (Panel B). Values (means± SE; n=6-8/group) with different letters are significantly different ($p<0.05$).

Figure 4 - Carnitine palmitoyltransferase-1 (CPT-1) activities in isolated mitochondria from red gastrocnemius muscle (panel A) and liver (panel B). CPT-1 activity was measured in the absence (black bars) or presence of an inhibitory amount of malonyl Co-A (malonyl Co-A 10 µM, light grey bars), and expressed as the difference between the malonyl Co-A doses (delta, open bars). Data is normalized to 1 in WL5 group and then expressed relative to WL5. Values (means± SE; n=6-8/group) with different letters are significantly different ($p<0.05$).

Figure 5 – PGC-1α mRNA in red gastrocnemius (panel A), liver (panel B), and epididymal adipose tissue (panel C). Data is normalized to 18S expression and expressed fold difference (means± SE; n=6-8/group). Values with different letters are significantly different ($p<0.05$).

Table 1

DEXA Measurements		Group WL5	WL173	SED
Body Fat %	7 Days Prior to Sac	4.1±0.5^{A}	4.7±0.6^{A}	6.6±0.4^{B}
	Day of Sac	4.5±0.4^{A}	6.8±0.4^{B}	6.8±0.7^{B}
	Δ	0.4±0.4	2.1±0.8$^{\#}$	0.2±0.6
Total Body Fat (g)	7 Days Prior to Sac	9.1±1.0^{A}	10.7±1.7^{AB}	13.8±1.1^{B}
	Day of Sac	10.6±0.8^{A}	15.6±1.2^{B}	15.6±2.1^{B}
	Δ	1.5±0.8	4.9±1.7$^{\#}$	1.8±1.3
Total Lean Mass (g)	7 Days Prior to Sac	214.7±4.6	211.1±8.5	193.4±8.4
	Day of Sac	223.6±4.1	212.1±7.4	208.3±7.1
	Δ	8.9±2.8$^{\#}$	0.9±3.1	14.9±1.5$^{\#}$
Total Body Mass (g)	7 Days Prior to Sac	223.9±4.4	221.8±9.5	207.2±9.2
	Day of Sac	234.1±4.0	227.7±8.0	223.8±8.6
	Δ	10.3±2.4$^{\#}$	5.9±2.0$^{\#}$	16.6±1.5$^{\#}$
Ave Daily Food Intake (g)	Week -2 to -1	18.3±0.8^{A}	18.8±0.6^{A}	13.7±0.3^{B}
	Week -1 to 0	19.3±0.3^{A}	16.6±0.3$^{\#B}$	15.2±0.6$^{\#B}$

Table 1. Body composition and food intakes. Body anthropometric measurements were taken 7 days prior to sacrifice and on the day of sacrifice by small animal DEXA. Values are means ± SE (n=7-8/group). Δ, is the difference between the two measures. Weekly food intakes were made for the 2 weeks preceding sacrifice. Different letters indicate that values are significantly different between groups at 7 days prior to, or on the day of sacrifice ($p<0.05$). $^{\#}$ indicates a significant increase during the seven-day period within the same group ($p<0.05$). Daily food intake was averaged for the week prior to sacrifice (week -1 to 0) in WL5 and SED, and from two weeks prior to sacrifice to one week prior to sacrifice (week -2 to -1) in WL173, allowing comparison of pre WL food intake to post WL in the WL173 group. Values are means ± SE. WL5, WL173, and SED groups 7-8 animals.group.

Table 2.

Epididymal Fat Pad Characteristics	WL5	WL173	SED
Epididymal Fat Pad (g/g of BW*100)	5.13±0.24^{A}	6.84±0.38^{B}	6.63±0.42^{B}
Epididymal Fat Pad (g)	1.20±0.06	1.57±0.13	1.50±0.15
Serum Measurements	**WL5**	**WL173**	**SED**
Triacylglycerol (mg/mL)	34.3±2.4	44.8±3.3	38.3±4.2
Free fatty acids (mmol/L)	146.2±9.7	168.6±9.4	176.3±13.7
Glucose (mg/dL)	470.9±10.6	462.8±19.7	429.9±13.7

Table 2. Epididymal adipose and serum measurements. Values are means ± SE (n=6-8/group. Different letters indicate that values are significantly different between groups ($p<0.05$). Values are means ± SE.

Table 3 - Enzyme Activities

		WL5	WL173	SED
Red Gastrocnemius				
β-HAD	(nmol/min/ug)	26.5±3.7	26.6±2.6	23.1±2.0
Citrate synthase	(nmol/min/ug)	1786±184	1908±270	1342±141
Cytochrome c oxidase	(Units/min/g)	75.1±6.9	78.8±8.5	65.5±10.3
Liver				
β-HAD	(nmol/min/ug)	16.6±1.2	18.3±1.7	16.7±0.7
Citrate synthase	(nmol/min/ug)	408±25	376±32	374±15
Cytochrome c oxidase	(Units/min/g)	46.6±1.9	42.7±2.6	43.7±1.4
Epididymal Adipose				
β-HAD	(nmol/min/ug)	U.D.	U.D.	U.D.
Citrate synthase	(nmol/min/ug)	21±7	20±4	21±6
Cytochrome c oxidase	(Units/min/g)	0.7±0.1	1.0±0.2	0.9±0.2

Table 3 – Enzyme activities in red gastrocnemius, liver, and epididymal adipocytes. Values are means ± SE (n= 6-8/group). No significant differences between groups were detected. β-HAD = β-hydroxyacyl CoA dehydrogenase; U.D. = Undetectable.

Figure 1

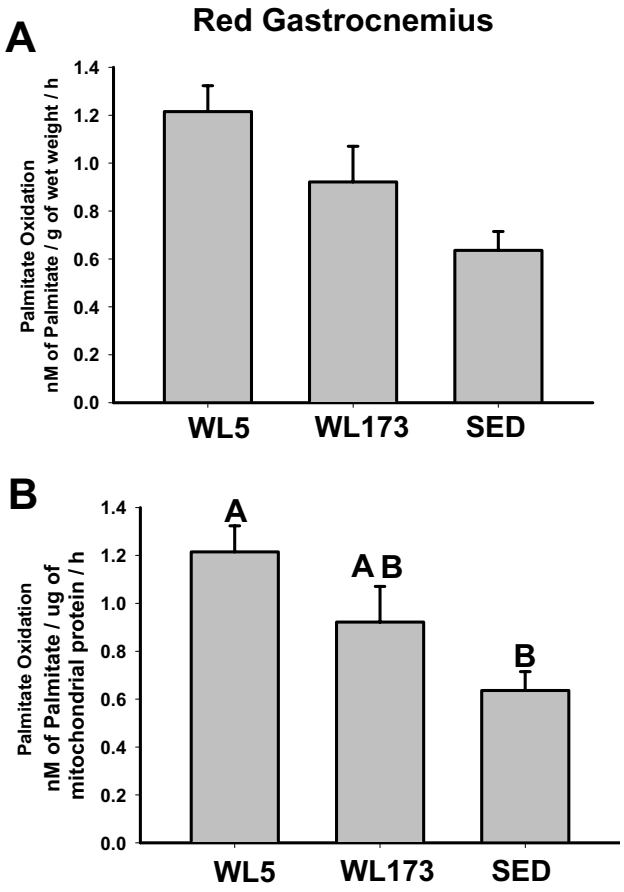

A
Red Gastrocnemius
Palmitate Oxidation
nM of Palmitate / g of wet weight / h
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WL5
WL173
SED
B
Palmitate Oxidation
nM of Palmitate / ug of
mitochondrial protein / h
A
A B
B
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WL5
WL173
SED

Figure 2.

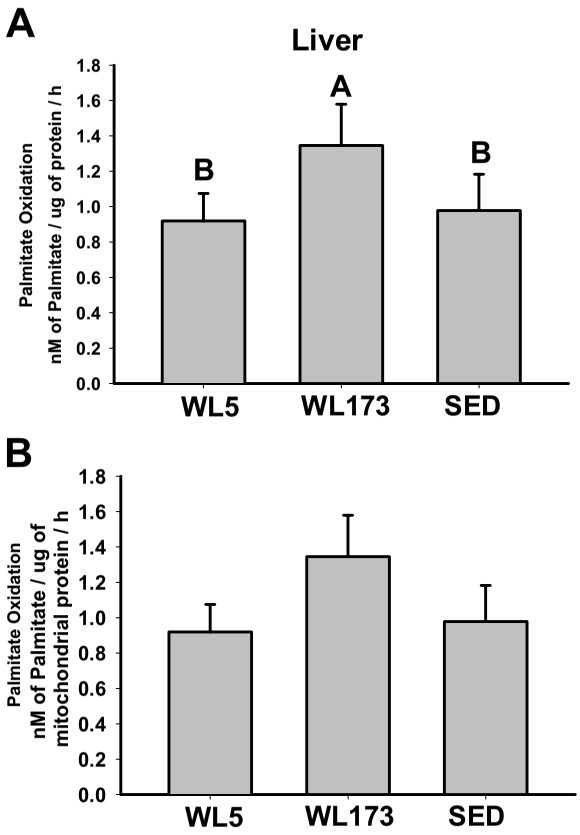

A
Liver
Palmitate Oxidation
nM of Palmitate / ug of protein / h
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
B
A
B
WL5
WL173
SED
B
Palmitate Oxidation
nM of Palmitate / ug of
mitochondrial protein / h
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WL5
WL173
SED

Figure 3

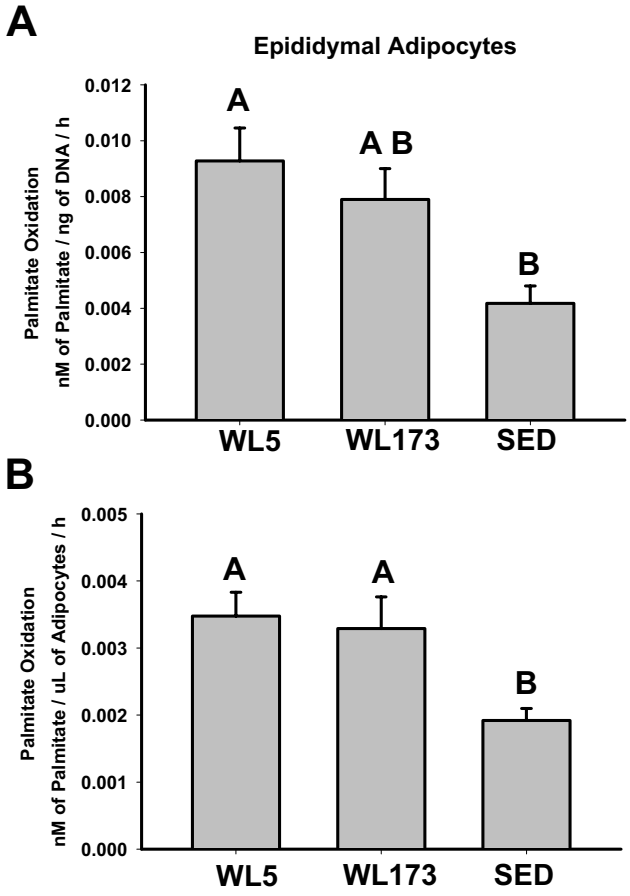

A
Epididymal Adipocytes
Palmitate Oxidation
nM of Palmitate / ng of DNA / h
0.012
0.010
0.008
0.006
0.004
0.002
0.000
A
A B
B
WL5
WL173
SED
B
Palmitate Oxidation
nM of Palmitate / uL of Adipocytes / h
0.005
0.004
0.003
0.002
0.001
0.000
A
A
B
WL5
WL173
SED

Figure 4

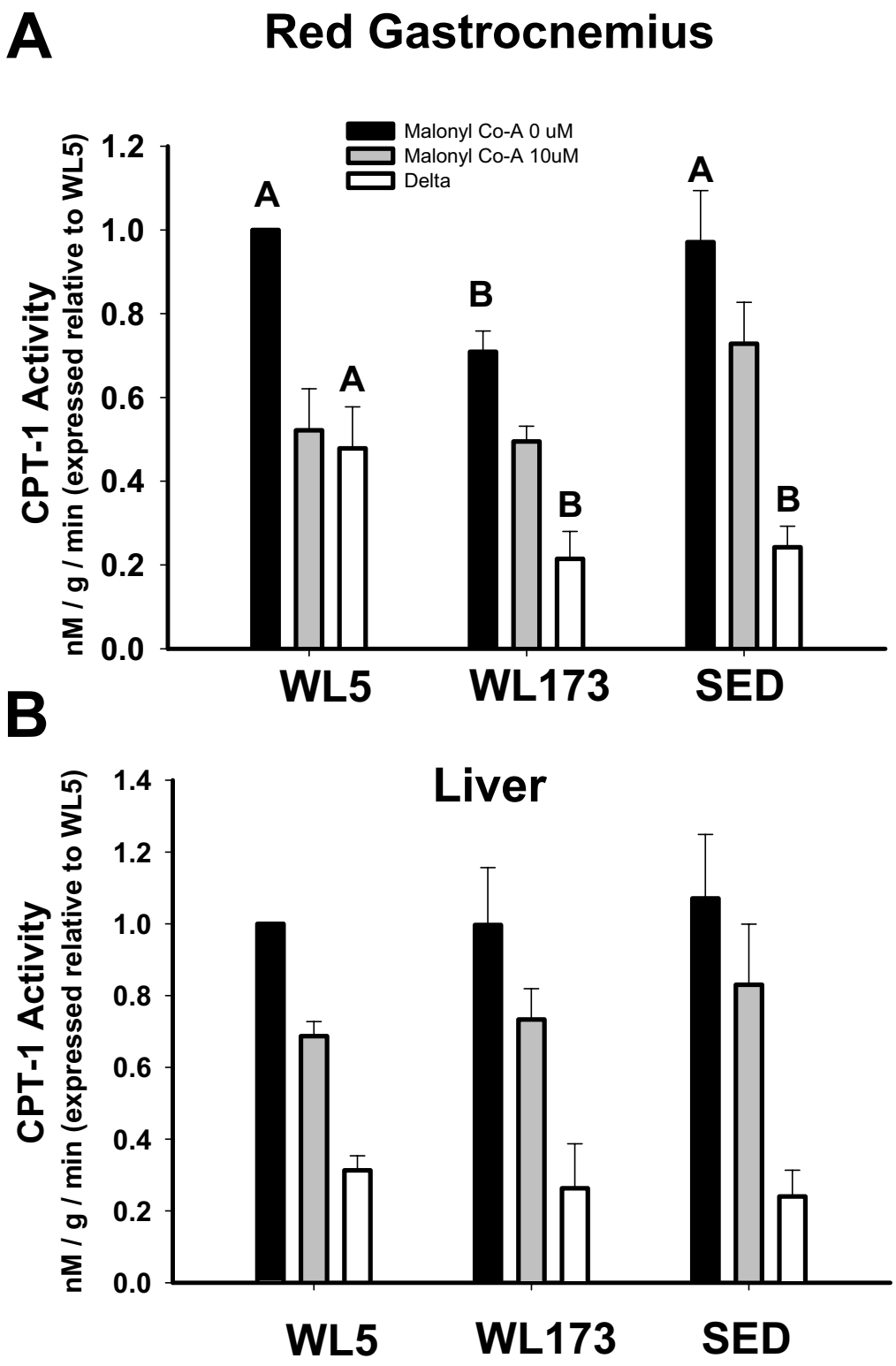
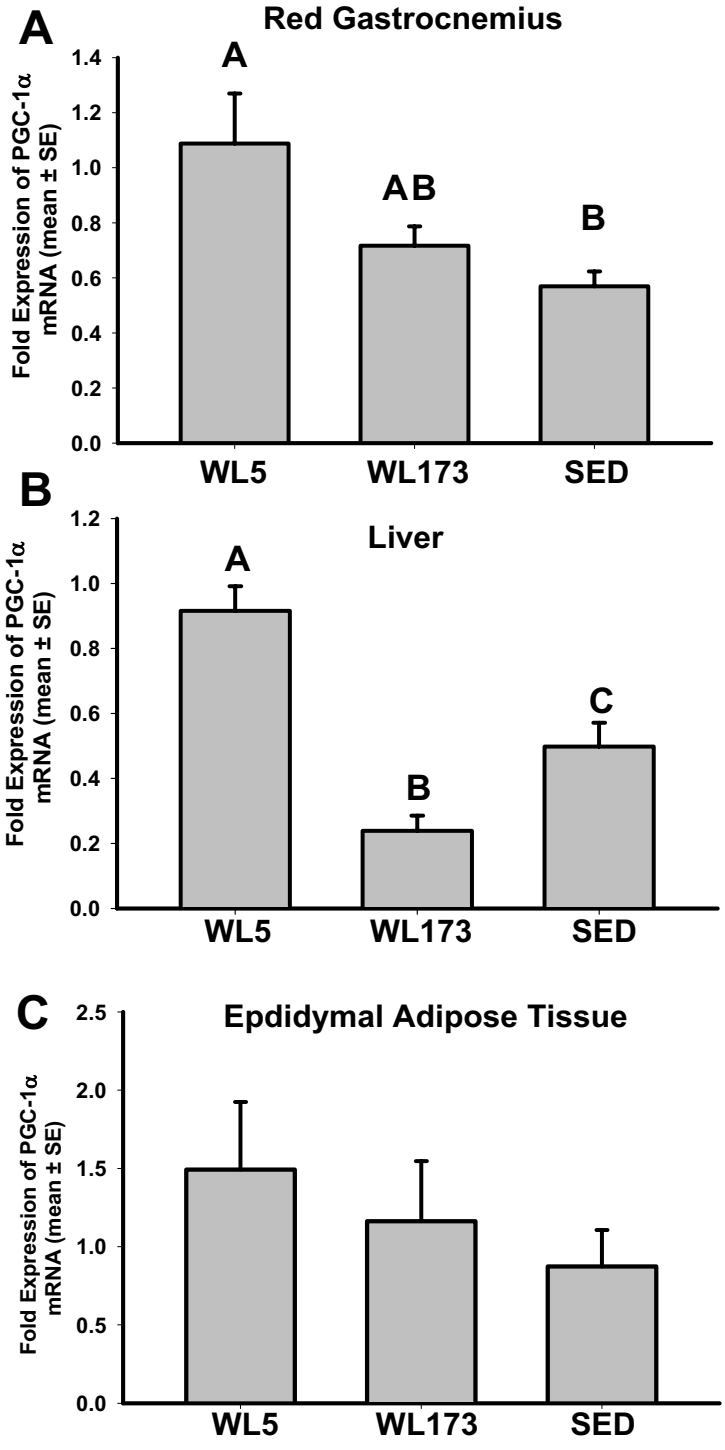

A
Red Gastrocnemius
Malonyl Co-A 0 uM
Malonyl Co-A 10uM
Delta
CPT-1 Activity
nM / g / min (expressed relative to WL5)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
A
B
B
A
B
WL5
WL173
SED
B
Liver
CPT-1 Activity
nM / g / min (expressed relative to WL5)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
WL5
WL173
SED

Figure 5


A
Red Gastrocnemius
Fold Expression of PGC-1α mRNA (mean ± SE)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
AB
B
WL5
WL173
SED
B
Liver
Fold Expression of PGC-1α mRNA (mean ± SE)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
A
B
C
WL5
WL173
SED
C
Epdidymal Adipose Tissue
Fold Expression of PGC-1α mRNA (mean ± SE)
2.5
2.0
1.5
1.0
0.5
0.0
WL5
WL173
SED