 Journal of Pediatric Endocrinology & Metabolism, 22, 409-415 (2009)

School-based Exercise Improves Fitness, Body Composition, Insulin Sensitivity, and Markers of Inflammation in Non-Obese Children

Aaron L. Carrel[1], Jennifer J. McVean[1], R. Randall Clark[2], Susan E. Peterson[1], Jens C. Eickhoff[1] and David B. Allen[1]

Department of Pediatrics, University of Wisconsin Children's Hospital[1] and Sports Medicine[2], Madison, WI, USA

ABSTRACT

Background: **Poor cardiovascular fitness (CVF) is a risk factor for obesity, as well as insulin resistance (IR), inflammation, and cardiovascular disease. We have previously shown that a school-based fitness curriculum can improve CVF, as well as IR and body composition in obese children. Whether such a program improves CVF, IR, and other health indicators in non-obese children is unresolved.**

Aim: **To determine whether a school-based fitness program improves body composition, CVF, markers of inflammation (e.g. CRP, TNF-α, adiponectin), and insulin sensitivity in non-obese children.**

Study design: **35 non-obese middle school children with body mass index below the 95th percentile for age were enrolled in a 'fitness-oriented' gym class. Children underwent fasting evaluation of insulin, glucose, adiponectin, CRP, TNF-α, body composition by dual X-ray absorptiometry (DXA), and maximal VO_2 treadmill testing at baseline (prior to the school year) and again at end of the school year.**

Main outcome measures: **Testing for CVF (maximal VO_2 treadmill testing), DXA, and fasting evaluation of insulin, glucose, adiponectin, CRP and TNF-α.**

Results: **Children demonstrated a decrease in BMI z-score (-0.14 ± 0.33, p = 0.02), HOMA-IR (-0.15 ± 0.35, p = 0.016), and TNF-α (-2.55 ± 1.79 pg/ml, p <0.001), and an increase in VO_{2max} (+1.58 ± 2.34 ml/kg/min, p <0.001), adiponectin (+7,553 ± 11,100 ng/ml, p <0.001), and muscle mass (+2,282 ± 1,882.73 g, p <0.001) after nine months of study.**

Conclusions: **The school-based fitness oriented curriculum resulted in improved body composition and insulin sensitivity, increased CVF, and decreased inflammation in non-obese children. Combined with prior studies, these data demonstrate that school-based fitness curricula can benefit both obese and non-obese children. Partnerships with schools to promote fitness should be part of a public health approach to improving children's health.**

KEY WORDS

schools, insulin resistance, VO_{2max}

Reprint address:
Aaron L. Carrel, M.D.
Department of Pediatrics
University of Wisconsin Children's Hospital
600 Highland Ave. H4-436
Madison, WI 53792, USA
e-mail: alcarrel@wisc.edu

INTRODUCTION

Poor physical fitness, as well as obesity, are risk factors for insulin resistance (IR), type 2 diabetes mellitus (DM2), inflammation and cardiovascular disease[1-3]. In adults, poor cardiovascular fitness (CVF) (as measured by maximal-VO_2 uptake) is a risk factor for IR and inflammation, independent of obesity[4]. Given the multiple factors contributing to the current epidemic of childhood obesity, an effective strategy for the prevention and treatment of childhood obesity must be pervasive and collaborative in its scope. One attractive venue for such a collaborative effort is the school setting.

While obesity increases the risk of IR, DM2, inflammation and other cardiovascular morbidities[5], it has been demonstrated in adults that *fitness level* is a stronger predictor of mortality than obesity[6].

It is thought that the beneficial effect of fitness training on insulin sensitivity reflects the combined effects of increased lean mass and reduced fat mass[7]. However, physical activity alters insulin sensitivity *independent of changes in weight and body composition* in adults[8] and in obese children[9,10]. We have shown that, in obese children, both CVF, as measured by maximal VO_2, and body fat were highly significant independent predictors of insulin sensitivity[11]. These data indicate that efforts to improve IR and health in children should include a focus on increasing physical activity, in addition to restricting calories for weight management[12].

We have previously reported that a school-based intervention for obese children was effective at improving CVF, and decreasing percent body fat and fasting insulin[13]. However, whether these beneficial changes would also occur in *non-obese* children is unclear. Most adults do not achieve the Surgeon General's recommendations for moderate physical activity[14]. Thus, childhood has been identified as a critical period for nurturing lifetime activity behavior and school physical education as a vehicle to promote active lifestyles[16,17]. We hypothesized that beneficial changes could also occur in non-obese children from a school-based program.

Encouraging additional exercise in children can be promoted or obstructed in varying environments[15-17]. The most successful programs are those that incorporate activity into the child's lifestyle, as a part of the family and school environment[18-20]. Gutin *et al.* have successfully designed school-based programs that promote physical activity and monitor changes in fitness, as well as metabolic parameters[18]. Jamner *et al.* demonstrated that a school-based intervention in adolescent females can increase physical activity and prevent a decline in cardiovascular fitness[21]. Some school-based nutrition and exercise interventions, such as 'Planet Health', have been successful at reducing body mass index (BMI) and triceps skinfold thickness among female students[22,23] while others (e.g. the 'Pathways' project) have fallen short of their goals in Native American schools[24]. Our study differs from previous interventions by focusing upon *fitness*, and its effect on levels of fasting insulin and inflammation, rather than weight or BMI, in exclusively 'non-obese' children.

CHILDREN AND METHODS

Thirty-five children with BMI below the 95th percentile for age were invited for participation in this study. Children were evaluated for 'baseline testing' at the University of Wisconsin Exercise Science Laboratory (ESL). All testing was completed by the same investigators, during a single visit following an overnight fast. The PI had full access to all the data and takes responsibility for the integrity of the data and data analysis. The procedures were approved by the Human Subjects Committee, and informed written consent was obtained prior to initiating the testing protocol. Testing included blood work to determine fasting glucose and insulin levels, adiponectin, CRP and TNF-α, baseline body composition, and CVF assessment prior to beginning the program. Height was measured on a wall-mounted stadiometer to the nearest 0.1 cm. Weight was measured on a calibrated beam balance platform scale to the nearest 0.1 kg.

Percent body fat and fat free mass (FFM) were measured by DXA. Whole body scans were performed using the Norland XR-36 whole body bone densitometer (Norland Corporation, Ft. Atkinson, WI, USA) and tissue masses were analyzed using software version 3.7.4/2.1.0. Children removed metal objects or clothing containing metal components and wore only workout shorts and a T-shirt for the scan procedure, methods described previously[25]. Each scan session was preceded by a calibration routine using multiple quality control phantoms that simulate soft tissue and bone. Based on 18 scans of six children using the XR-36 whole body procedures, the total body coefficients of variation (CV) were as follows: soft tissue mass 0.2%, total body mass 0.2%, lean body mass 1.0%, fat mass 2.5%, percent fat 2.4% and total bone mineral content (BMC) 0.9%.

Children underwent measurement of maximal oxygen consumption (VO_{2max}) performed by open circuit spirometry using a progressive treadmill walking protocol to volitional fatigue using a Medical Graphics CPX-D (St. Paul, MN). Requirements to ensure children reached their maximal oxygen consumption by this protocol included at least two of the following three criteria: 1) maximal heart rate >200 beats per minute; 2) respiratory exchange ratio (VCO_2/VO_2) >1.0; and 3) a plateau in oxygen consumption. All children reached their maximal oxygen consumption according to the above criteria.

A 10 ml fasting blood sample for insulin, glucose, adiponectin, C-reactive protein (CRP) and TNF-α was obtained from an antecubital vein. Samples were separated by low speed centrifugation at 4000 *g* for 10 minutes. Fasting insulin concentration was determined using a chemiluminescent assay (Esoterix, Calabasas Hills, CA) and glucose concentration was determined by an enzymatic method (Beckman Diagnostics, Fullerton, CA). Adiponectin was measured by radioimmunoassay (Northwest Lipid Research Laboratories, Seattle, WA). High sensitivity CRP was measured immunochemically using the Dade-Behring reagent on a nephelometer autoanalyzer (Northwest Lipid Research Laboratories, Seattle, WA).

Analysis of TNF-α was performed on a BioRad Bioplex instrument using a Human Serum Adipokine Panel B kit from Linco Research Inc. (Northwest Lipid Laboratories, Seattle, WA).

Study design

Once baseline testing was completed, children were enrolled into lifestyle focused fitness oriented gym classes for 9 months (the entire school year). Participants were in classes designed to focus on lifelong fitness, and make fitness fun and achievable and maximize the amount of movement during the class period. At the end of the school year, post-treatment assessment of all study outcomes was obtained. The study was designed so that each participant served as his/her own control. With a planned sample size of 35 children participating in the 'fitness-oriented' gym class, the study had >80% power to detect a difference of half a standard deviation (SD) between baseline and post-treatment assessment in body composition, insulin sensitivity and CVF parameters, assuming a two-sided 5% significance level.

School fitness curriculum

The frequency of fitness-oriented physical education classes was five times every 2 weeks for a 45-minute class period. All classes took place within the normal school day. The curriculum was modified to encourage student participation. Competitive games were de-emphasized and replaced with lifestyle-focused activities (walking, cycling, snowshoeing). A consistent warm-up plan brought students into movement participation as quickly as possible soon after they entered the gym. Skills were taught with the class broken down into small groups promoting more movement and less 'watching others' time. The ethos of the class encouraged physical fitness, less self-conscious focus on appearance and full group participation.

Statistical analyses

Categorical variables were summarized using frequencies and percentages. All continuous variables were summarized and reported in terms of means ± standard deviations (SD). Changes from baseline were evaluated using a paired t-test or the non-parametric Wilcoxon Signed Rank test, if data were not normally distributed. Deviation of normality was assessed using probability plots and the Shapiro-Wilk test. All p-values were two-sided, and $p < 0.05$ was used to indicate statistical significance. Due to the sample size, exact p values were calculated for all non-parametric tests. Statistical analyses were performed using SAS software version 8.2 (SAS Institute, Cary, NC, USA).

RESULTS

Patient characteristics and study results are presented in Tables 1-3 as means ± SD.

Anthropometrics and body composition

At baseline, the mean age of the study participants was 12.3 ± 0.5 years (range 11-13), and 83% of them were female. The mean BMI was 20.7 ± 2.7 (range 15.4-24.1; BMI z-score range -1.51-1.63). In this fitness oriented class there was a decrease in BMI z-score (-0.14 ± 0.33, p = 0.022),

TABLE 1

Baseline demographics (means ± SD)

	Total (n = 35)
Age (years)	12.3 ± 0.5
Body mass index	20.7 ± 2.7
VO_{2max} (ml/kg/min)	40.1 ± 5.4
% body fat	27.6 ± 6.4
Fasting insulin (μU/ml)	11.9 ± 4.5
Fasting glucose (mg/dl)	94.8 ± 6.3

TABLE 2

Change from baseline evaluation (n = 35)

	Mean ± SD	**p value**
Body mass index z-score	-0.14 ± 0.33	0.022
Muscle mass (g)	2,282 ± 1,883	<0.001
Fat mass (g)	792 ± 2,629	0.004
VO_{2max} (ml/kg/min)	1.58 ± 2.34	<0.001
Fasting glucose (mg/dl)	-2.8 ± 6.4	0.014
HOMA-IR	-0.15 ± 0.35	0.016
TNF-α (pg/ml)	-2.55 ± 1.79	<0.001
Adiponectin (ng/ml)	7,553 ± 11,100	<0.001

TABLE 3

Sub-analysis of participants with BMI <85^{th} percentile (n = 22), change from baseline

	Mean ± SD	**p value**
Body mass index	-0.53 ± 0.74	0.35
Muscle mass (g)	2,073 ± 1,934	<0.001
Fat mass (g)	1,342 ± 1,362	<0.001
VO_{2max} (ml/kg/min)	1.8 ± 2.5	0.002
Fasting glucose (mg/dl)	-4.1 ± 6.6	0.011
HOMA-IR	-0.22 ± 0.36	0.013
TNF-α (pg/ml)	-1.98 ± 1.90	0.001
Adiponectin (ng/ml)	5,808 ± 11,229	0.029
C-reactive protein (mg/dl)	0.08 ± 0.20	0.013

and an increase in muscle mass (2,281.9 ± 1,882.7 g, $p < 0.001$) after completion of the 9-month intervention. The results are summarized in Table 2.

Cardiovascular fitness

After 9 months of the fitness intervention, the group showed significant improvements in CVF (VO_{2max}) compared to baseline measurements (+1.6 ± 2.3 ml/kg/min, $p < 0.001$).

Insulin sensitivity

At baseline, mean fasting insulin was 12.0 ± 4.5 µIU/ml (normal values 4-24 µIU/ml) and HOMA-IR was 2.83 ± 1.2. After 9 months, children showed significant improvements in mean fasting glucose (-2.8 ± 6.4 mg/dL, $p = 0.014$), and insulin sensitivity measured by the homeostasis model (HOMA) (-0.15 ± 0.35, $p = 0.016$).

Markers of inflammation

Participants showed a significant decrease in TNF-α (-2.55 ± 1.79 pg/ml, $p < 0.001$) and a significant increase in adiponectin (7,553 ± 11,100 ng/ml, $p < 0.001$) after nine months.

Anthropometrics, body composition, cardiovascular fitness, insulin sensitivity and markers of inflammation in participants with BMI <85th percentile

Analysis of the subgroup of children with BMI less than the 85th percentile (normal weight; $n = 22$) showed a non-significant decrease in BMI z-score of -0.5 ($p = 0.35$), with an increase in both muscle mass and fat mass, over the nine-month intervention period (see Table 3). Nevertheless, peak VO_2 still improved in this subgroup (137 ± 155 l/min; $p < 0.001$) as did fasting glucose and insulin sensitivity as measured by HOMA-IR. Indicators of inflammation showed inconsistent results, with a decrease in TNF-α and increase in adiponectin, but an increase in CRP.

DISCUSSION

It is generally acknowledged that change in health behavior is facilitated when interventions combine focus on both the individual and the environment (e.g. school)[26]. Previously, we demonstrated health benefits including improved CVF and IR from a school-based fitness curriculum in *obese* children. This study evaluated the effect of a school-based fitness program with respect to fitness, insulin sensitivity and body composition in *non-obese* middle school children. These results demonstrate that body composition, CVF (VO_2), insulin sensitivity and markers of inflammation were favorably changed in *non-obese* children who experienced fitness-emphasis modifications of the school physical education curriculum. Even this small change in the amount of physical activity (five sessions every 2 weeks) showed beneficial health effects. Similar benefits have been shown following lifestyle improvements in adults with known glucose intolerance[27].

Despite evidence of the association between CVF and IR in children[11], questions remain whether target 'healthy' criteria values for CVF can be identified and how such standards for fitness should influence public health policy[28]. It is important to note that the most significant changes noted in our program, changes in CVF (as measured by maximal O_2 consumption during treadmill testing to voluntary exhaustion), have not routinely been measured in most previous programs. Further, given the expected and desired normal growth of middle-school children, we specifically did not use weight as a primary endpoint of this intervention. We acknowledge that there are many ways of evaluating health and fitness, including maximal VO_2 testing, body composition, and insulin sensitivity. In this and prior studies, we have demonstrated improved BMI z-score and insulin sensitivity in spite of an increase in total body fat. Thus, there is growing evidence that, as in adults, physical activity and fitness, as well as fatness, are both important in affecting health in children.

While it has been shown that aerobic exercise is useful as a treatment strategy for insulin resistance[27], it is important to consider what changes in fitness levels would normally be expected for children. In a longitudinal study of fitness in 8-10 year olds, data suggest that fitness levels tend to remain constant without formal intervention, but that children with obese parents tend to have less physical activity and lower fitness levels[29,30]. In children and adolescents, percent body fat and visceral adipose tissue are also positively correlated with IR[31], an independent predictor of stroke, cancer, coronary artery disease, hypertension and DM2 in adulthood[7,32]. Our group has previously shown that CVF is a stronger predictor of fasting insulin levels than fatness in overweight middle school children[11].

Puberty is associated with an increase in insulin resistance, and in susceptible children represents a period of increased risk for obesity and DM2. If puberty were to influence the results of this study, one might assume that 9 months after starting this intervention, insulin sensitivity would have decreased as the children matured. It is noteworthy, therefore, that the opposite occurred in the study participants, i.e. improved insulin sensitivity after completing the intervention. Consequently, while it is difficult to factor the possible influences of pubertal advancement on IR in this cohort, an improvement in insulin sensitivity during advancing adolescent age argues strongly for a salutary effect of increased physical activity.

The limitations of this study include the lack of a formal control group, the relatively small sample size, the lack of direct pubertal staging, and a small number of non-Caucasian children. This project focused on increasing physical activity and no dietary intervention or outcomes were measured.

As childhood obesity is predictive of adult obesity[5], it is important to develop and evaluate interventions that begin during childhood. This study demonstrates that it is feasible to achieve changes in physical activity sufficient to favorably affect fitness level, percent body fat, insulin sensitivity, and markers of inflammation in non-obese children. These findings can provide encouragement to primary care physicians, public health researchers, and school personnel that increases in physical activity have tangible benefits, and should encourage the development of fitness-emphasis physical education programs. Optimally, an effective public health approach would also promote increased physical activity outside of school and throughout the summer months[33], as physical activity recommendations cannot be met through school-year physical education classes alone.

CONCLUSIONS

School-based fitness programs can significantly improve cardiovascular fitness levels, insulin sensitivity, markers of inflammation, and body composition in non-obese children. These findings suggest that modifications of school physical education curricula toward a fitness emphasis may be an effective vehicle for increasing physical activity and improving cardiovascular health for all children.

ACKNOWLEDGEMENTS

We thank Lori Clark MS, Peg Guse and the administration and students of Hamilton Middle School, Madison, WI. This study was supported by the UW Sports Medicine Classic Fund.

REFERENCES

1. Katzmarzyk PT, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C. Fitness, fatness, and estimated coronary heart disease risk: the HERITAGE Family Study. Med Sci Sports Exerc 2001; 33: 585-590.
2. Must A, Strauss RS. Risks and consequences of childhood and adolescent obesity. Int J Obes Relat Metab Disord 1999; 23 (Suppl 2): S2-11.
3. Freedman DS, Srinivasan GL, Burke CL, Shear CL, Smoak C, Harsha D, et al. Relation of body fat distribution to hyperinsulinemia in children and adolescents. The Bogalusa Heart Study. Am J Clin Nutr 1987; 46: 403-410.
4. Blair SN, Cheng Y, Holder JS. Is physical activity or physical fitness more important than defining health benefits? Med Sci Sports Exerc 2001; 33: S379-S399.
5. Dietz WH. Health consequences of obesity in youth: Childhood predictors of adult disease. Pediatrics 1998; 101: 518-525.
6. Lee CD, Blair SN, Jackson AS. Cardiorespiratory fitness, body composition, and all-cause and cardiovascular disease mortality in men. Am J Clin Nutr 1999; 69: 373-380.

7. Sinha R, Dufour S, Petersen KF, LeBon V, Enoksson S, Shulman GI, et al. Assessment of skeletal muscle triglyceride content by ^{1}H nuclear magnetic resonance spectroscopy in lean and obese adolescents: relationships to insulin sensitivity, total body fat, and central adiposity. Diabetes 2002; 51: 1022-1027.
8. Kelley DE, Goodpaster BH. Effects of physical activity on insulin action and glucose tolerance in obesity. Med Sci Sports Exerc 1999; 31 (Suppl): S619-623.
9. Travers SH, Labarta JI, Gargosky SE, Rosenfield RG, Jeffers BW, Eckel RH. Insulin-like growth factor binding protein-1 levels are associated with insulin sensitivity and obesity in prepubertal children. J Clin Endocrinol Metab 1998; 83: 1935-1939.
10. Eliakim A, Scheet T, Newcomb R, Cooper DM. Fitness training and the growth hormone/IGF-1 axis in prepubertal girls. J Clin Endocrinol Metab 2001; 86: 2797-2802.
11. Allen DB, Clark RR, Peterson SE, Nemeth BA, Eickhoff J, Carrel AL. Fitness is a stronger predictor of fasting insulin than fatness in overweight male middle-school children. J Pediatr 2007; 150: 383-387.
12. Dietz WH. Childhood weight affects adult morbidity and mortality. J Nutr 1998; 128 (Suppl): 411S-414S.
13. Carrel AL, Clark RR, Peterson SE, Nemeth BA, Sullivan JC, Allen DB. School-based exercise program improves fitness, body composition and insulin sensitivity in overweight children: a randomized, controlled study. Arch Pediatr Adolesc Med 2005; 159: 963-968.
14. United States Department of Health and Human Services. Healthy People 2010: Understanding and Improving Health. Washington, DC: Government Printing Office, 2000; pp 1272.
15. Knowler WC, Narayan KMV, Hanson RL. Preventing non-insulin dependent diabetes. Diabetes 1995; 44: 483-488.
16. Owens S, Gutin B. Exercise testing of the child with obesity. Pediatr Cardiol 1999; 20: 79-83.
17. Epstein LH, Valoski A, Wing RR. Ten year outcomes of behavioral based family treatment for childhood obesity. Health Psychol 1994; 13: 373-383.
18. Gutin B, Barbeau P, Owens S, Lemmon CR, Bauman M, Allison J. Effects of exercise intensity on cardiovascular fitness, body composition, and visceral adiposity in obese adolescents. Am J Clin Nutr 2002; 75: 818-826.
19. Tuomilehto J, Lindstrom J, Eriksson J, Valle T. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. N Engl J Med 2001; 344: 1343-1350.
20. Sallis JF, McKenzie TL, Alcaraz JE, Kolody B, Faucette N, Hovell MF. Effects of a 2-year physical education program (SPARK) on physical activity and fitness in elementary school students. Sports, Play and Active Recreation for Kids. Am J Public Health 1997; 87: 1328-1334.
21. Jamner MS, Spruijt-Metz D, Bassin S, Cooper DM. A controlled evaluation of a school-based intervention to promote physical activity among sedentary adolescent females: project FAB. J Adolesc Health 2004; 34: 279-289.
22. Gortmaker SL, Peterson KE, Wiecha J. Reducing obesity via a school-based interdisciplinary intervention among youth (Planet Health). Arch Pediatr Adolesc Med 1999; 153: 409-418.
23. Robinson TN. Reducing children's television viewing to prevent obesity: a randomized controlled trial. JAMA 1999; 282: 1561-1567.
24. Caballero B, Clay T, Davis SM, Ethelbah B, Lohman T, Norman J, et al. Pathways: a school-based, randomized controlled trial for the prevention of obesity in American Indian schoolchildren. Am J Clin Nutr 2003; 78: 1030-1038.
25. Clark RR, Bartok C, Sullivan JC, Schoeller DA. Minimum weight prediction methods cross-validated by the four compartment model. Med Sci Sports Exerc 2004; 36: 639-647.
26. Swinburn B, Egger G, Raza F. Dissecting obesogenic environments: the development of a framework for identifying and prioritizing environmental interventions for obesity. Prev Med 1999; 29: 563-570.
27. Katzmarzyk PT, Leon AS, Wilmore JH, Skinner JS, Rao DC, Rankinin T, et al. Targeting the metabolic syndrome with exercise: evidence from the HERITAGE family study. Med Sci Sports Exerc 2003; 35: 1703-1709.
28. Ruiz JR, Ortega FB, Villa I, Hurtig-Wenlof A, Oja L, Sjostrom M. High cardiovascular fitness is associated with low metabolic risk score in children: the European Youth Heart Study. Pediatr Res 2007; 61: 350-355.
29. Treuth MS, Butte NF, Adolph AL, Puyay MR. A longitudinal study of fitness and activity in girls predisposed to obesity. Med Sci Sports Exerc 2004; 36: 198-204.
30. Baranowski T, Bouchard C, Bar-Or O, Bricker T, Heath G, Kimm SY, Malina R, Obarzanek E, Pate R, Strong WB, et al. Assessment, prevalence, and cardiovascular benefits of physical activity and fitness in youth. Med Sci Sports Exerc 1992; 24 (Suppl): S221-236.
31. Kang H-S, Gutin B, Barbeau P, Owens S, Lemmon CR, Allison J, et al. Physical training improves insulin resistance syndrome markers in obese adolescents. Med Sci Sports Exerc 2002; 34: 1920-1927.
32. Yip J, Facchini F, Reaven GR. Resistance to insulin mediated glucose disposal as a predictor of disease. J Clin Endocrinol Metab 1998; 83: 2773-2776.
33. Carrel AL, Clark RR, Peterson SE, Nemeth BA, Eickhoff J, Allen DB. Improvements in fitness from a school-based intervention are lost during the summer in overweight middle-school children. Arch Pediatr Adoles Med 2007; 161: 561-564.