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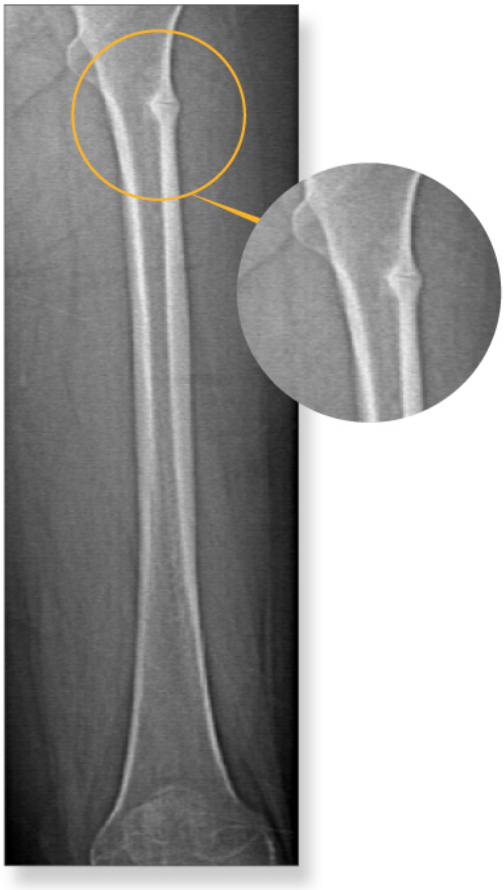

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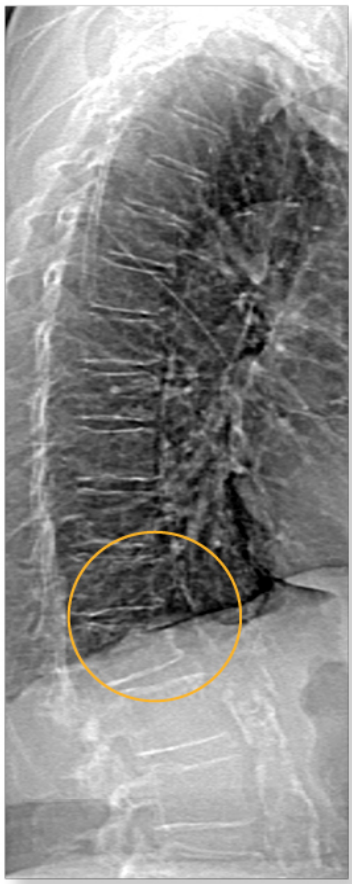

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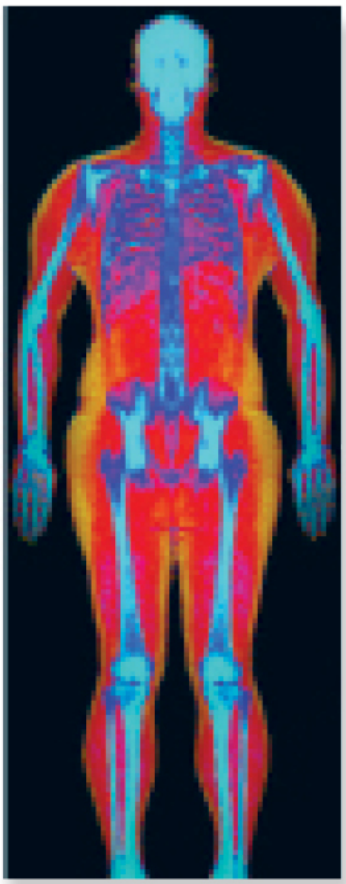

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Chronic Intravenous Aminobisphosphonate Therapy Increases High-Density Lipoprotein Cholesterol and Decreases Low-Density Lipoprotein Cholesterol

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ABSTRACT

Nowadays, bisphosphonates are considered the drugs of choice for the treatment of several bone disorders. Their exact mechanism of action is not clear but recently it has been reported that the aminobisphosphonates inhibit cholesterol biosynthesis and that this might be relevant for their actions on bone osteoclasts. The study includes 87 postmenopausal women with moderate to severe osteoporosis. The patients were randomly assigned to intravenous (iv) infusion of 50 mg of the aminobisphosphonate Neridronate dissolved in 100 ml of saline solution every 2 months for a year (44 patients). The remaining 43 served as controls. At the time of each infusion blood samples were obtained for the evaluation of total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C), apolipoprotein A-I (Apo A-I), apolipoprotein B (Apo B), and total and bone alkaline phosphatase (AP). Free deoxypyridinoline (f-DPD) was measured in fasting urine specimens. In the control group no significant changes were observed throughout the study period for any of the biochemical variables. In the Neridronate-treated patients both bone AP and f-DPD excretion fell significantly by 15–20%. In these patients serum total cholesterol and serum triglycerides showed marginal decreases, which were occasionally significant. LDL-C and Apo B fell by 5–6% and these changes were statistically significant at most time points. Apo A-I and HDL-C rose progressively with time. At the 12th month, HDL-C rose 17–18% ($p < 0.0001$) above the baseline values. Similar findings were obtained in four postmenopausal women given high iv doses of Pamidronate or Alendronate. In conclusion aminobisphophonates, at least when given iv, induce remarkable and unexpected effects on lipid metabolism with a final profile that might be clinically relevant. (J Bone Miner Res 2000;15:599–604)

Key words: **bisphosphonates, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, apolipoproteins, osteoporosis**

INTRODUCTION

BISPHOSPHONATES ARE a class of compounds resistant to metabolic degradation that have high affinity for bone mineral and the ability to inhibit osteoclast-mediated bone resorption.[1] These properties have made several bisphosphonates the drugs of choice for the treatment of several bone disorders such as Paget's disease, tumor osteolysis, and osteoporosis.[1–3] The bisphosphonates are taken up by several cells, and this is associated with a variety of intracellular effects, but the exact molecular mechanism of action has not been fully elucidated. Recently, it has been reported that several bisphosphonates inhibit squalene synthesis and cholesterol biosynthesis.[4] This effect can be observed both in vitro and ex vivo only with the more potent nitrogen-containing bisphosphonates and it appears to explain the osteoclast and macrophage apoptosis associated with the exposure of these cells to aminobisphosphonates.[5] Other bisphosphonates, not containing amino groups such as clodronate and etidronate do not interfere with sterol biosyn-

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thesis and they are metabolized to a cytotoxic analogue of adenosine triphosphate (ATP), leading to target cell death.[4,6]

On the basis of these new findings on the metabolic activities of aminobisphosphonates we decided to investigate the effect on lipid metabolism during an ongoing phase 2 clinical trial on the skeletal effects of intravenous (iv) Neridronate (Abiogen, Pisa, Italy). This is an aminobisphosphonate that has been used in patients with Paget's disease and malignant hypercalcemia and that is structurally very similar to Pamidronate (Novartis, Switzerland) and Alendronate (MSD, USA), with the hydroxyl-side chain being represented by aminohexane rather than aminopropane or aminobutane, respectively.[7,8]

MATERIALS AND METHODS

Patients

The study includes 87 postmenopausal women aged 53–72 years, who had a lumbar spine bone mineral density below 2 SD of the mean values of normal premenopausal women. None of the recruited patients had taken androgen, estrogen, calcitonin, bisphosphonates, fluoride, pharmacologic doses of vitamin D, or lipid-lowering drugs within the previous 12 months. Nineteen patients were on hypotensive drugs including thiazide diuretics, which were used most often, but the dosage had been stable for at least 6 months and it did not change during the study follow-up. Three months before entering the study all women were instructed to keep to their usual diet but to take 500 mg calcium supplements if their calcium intake was lower than 1000 mg/day. Seven patients, in whom serum 25-hydroxy-vitamin D concentration was lower than 10 ng/ml, also were given 50,000–100,000 U of oral vitamin D in 2–4 doses over 2–4 weeks.

Forty-four of the patients were randomly assigned iv infusion of 50 mg Neridronate dissolved in 100 ml of saline. The remaining 43 served as controls and were given a saline infusion only in the first month. Originally,we decided to evaluate the lipid profile occasionally only after the first infusion, but an interim analysis of the safety biochemical data of the first 13 patients within 6–8 weeks led us to modify the original protocol by including the lipid measurements for at least a year.

All women gave written informed consent to their participation in the study, in accordance with the ethical principles stated in the Declaration of Helsinky. The research protocol and its amendments were approved by our local Ethic Committee.

Analytic procedures

Fasting blood samples were obtained immediately before the commencement of treatment (or follow-up for control subjects) and again at 2, 4, 6, 8, 10, and 12 months. Samples were stored at −80°C while awaiting analysis. Samples were batched and measured in one assay. Serum cholesterol was measured enzymatically using the Cobas Integra Roche analyzer (F. Hoffman-La Roche Ltd. Basilea, Switzerland). Analytical imprecision was 0.95% at a level of 3.87 mmol/liter and 0.96% at 9.49 mmol/liter. Serum triglycerides were assayed using the glyceryl dehydrogenase reaction after enzymatic hydrolysis of the glycerides on the Cobas Integra Roche analyzer. Analytical imprecision was 1.4% at a level of 1.49 mmol/liter and 1.5% at 5.19 mmol/liter. High-density lipoprotein cholesterol (HDL-C) was measured after precipitation of low-density lipoprotein cholesterol (LDL-C) and very low density lipoproteins (VLDL) with polyanions and phosphotungstic acid/magnesium chloride. The supernatant was assayed enzymatically on the Cobas Mira S analyzer (F. Hoffman-La Roche Ltd. Basilea, Switzerland) and had an analytical imprecision of 3.8% at a concentration of 1.03 mmol/liter per milligrams per deciliter and 3.75% at 3.98 mmol/liter. LDL-C was calculated using the Friedewald formula.[9] In none of the specimens were the triglycerides values greater than 10.33 mmol/liter. Apolipoprotein A-I (Apo A-I) and apolipoprotein B (Apo B) were measured on the Behring Nephelometer Analyzer (BNA) (Behringwerke, Marburg AG, Germany) employing anti-Apo A-I and anti-Apo B nephelometric antisera (Behringwerke, Marburg AG, Germany). Results were evaluated by the logit-log function of light-scattering intensities versus respective concentrations. The intra-assay and interassay CVs of Apo A-I and Apo B measurements were less than 3% and 5%, respectively. The total alkaline phosphatase (AP) was measured by standard autoanalyzer procedures (Cobas Integra Roche analyzer. The intra-assay and interassay CVs were below 1.6%. Serum bone AP by ELISA (Alkphase-B, Metra Biosystems, Mountain View, CA, U.S.A.). The intra-assay and interassay CVs were 3.9% and 7.6% respectively. Free deoxypyridinoline (f-DPD) was assayed in spot fasting urine samples by ELISA. Intra-assay and interassay CVs were less than 10%. The data were corrected by the urinary creatinine concentration measured by a standard colorimetric method.

Statistical analysis

The absolute and percent changes in biochemical markers of bone turnover and serum lipids were distributed normally. The significance of the differences from the baseline was then evaluated by Student's *t*-test for paired observation and the *p* values were adjusted by the Bonferroni test of multiple comparisons. The comparison of the changes between treated and control patients was evaluated by analysis of variance (ANOVA) for repeated measures and then by Student's *t*-test for unpaired observation (SPSS 8.0 by SPSS Inc, Chicago, IL, U.S.A.).

RESULTS

The baseline characteristics of the women in the Neridronate group and of the control group were similar and never statistically different (Table 1). All patients completed the 12-month study; they attended to the investigation controls diligently, did not modify their diet, and were not given other drugs that were able to interfere with lipid and bone metabolism. The distribution of concomitant therapies known to affect lipid profile was similar in the Neridronate group

TABLE 1. BASELINE CHARACTERISTICS OF THE PATIENTS BY GROUP (MEAN ± SE) INCLUDING BODY MASS INDEX (BMI, BODY WEIGHT/HEIGHT, KG/M^2), SERUM TOTAL AP, BONE AP, AND URINARY EXCRETION OF f-DPD

	Neridronate	*Controls*
No.	44	43
Age (years)	62.54 ± 1.44	63.30 ± 1.35
Years since menopause (ysm)	16.27 ± 1.64	15.53 ± 1.43
Body weight (kg)	61.61 ± 1.50	62.09 ± 1.67
Height (cm)	1.60 ± 0.01	1.59 ± 0.01
BMI (kg/m^2)	24.11 ± 0.54	24.88 ± 0.91
Total AP U/l (32-122)	65.63 ± 3.36	62.37 ± 3.02
Bone AP U/l (12-43)	15.16 ± 0.83	13.14 ± 0.81
f-DPD nM/mM Creat (3.0–7.4)	10.53 ± 1.00	9.89 ± 0.70

BMI, body mass index.

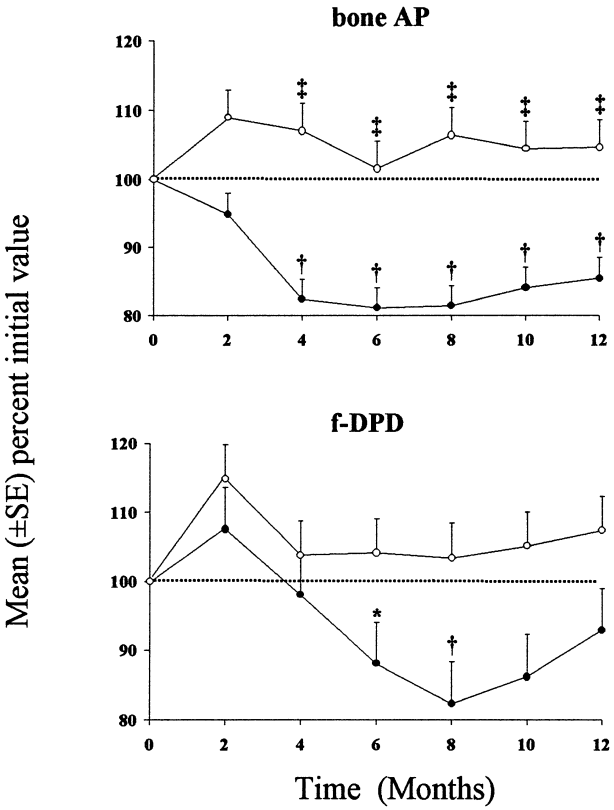

FIG. 1. Percent changes in serum total cholesterol and triglycerides in Neridronate-treated (closed circles) and control patients (open circles). The symbols (*$p < 0.05$; †$p < 0.01$) over the closed circles indicate the statistical significance of the changes versus baseline in the Neridronate patients (Student's *t*-test for paired observation) and those over the open circles (control patients) indicate the between-group differences (ANOVA for repeated measures).

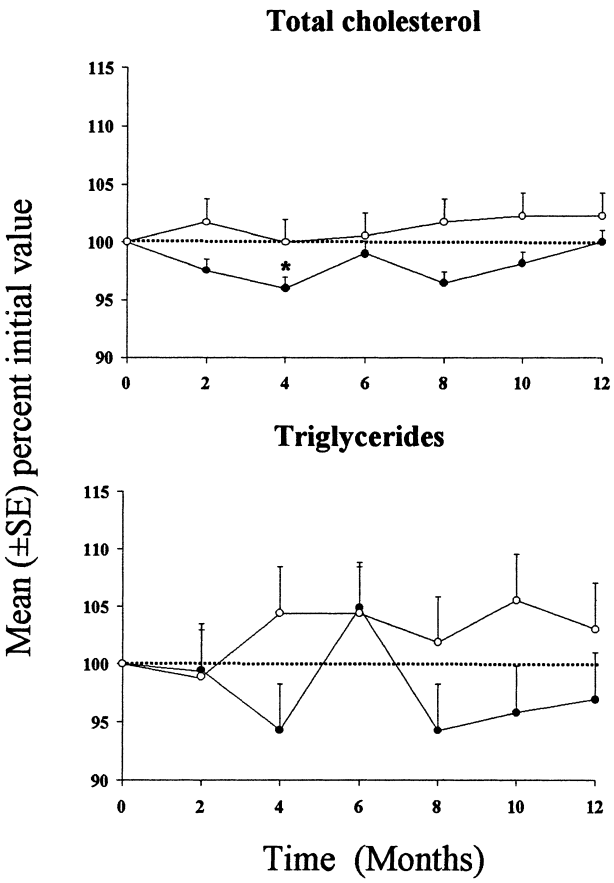

FIG. 2. Percent changes in serum lipoproteins in Neridronate-treated (closed circles) and control patients (open circles). The symbols (*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$) over the closed circles indicate the statistical significance of the changes versus baseline in the Neridronate patients (Student's *t*-test for paired observation) and those over the open circles (control patients) indicate the between-group differences (ANOVA for repeated measures).

and in the control group. Thiazides were taken by nine patients of both groups and angiotensin converting enzyme inhibitors were taken by five patients of the Neridronate group and by four patients of the control group. The physical activity and the mean body weight did not change during the study period.

In the control group the biochemical profile did not change (Table 2; Figs. 1 and 2). As compared with the control subjects the Neridronate group had a statistically significant decrease in bone AP (Fig. 3). Serum concentration of bone AP decreased during the first 4 months (−18% ± 22% SD) and remained suppressed thereafter. The changes in the urinary excretion of f-DPD were somewhat more erratic. In the 12th month the changes were significant only in absolute terms.

Mean serum cholesterol and serum triglycerides slightly decreased in the Neridronate group, but the changes with respect to the baseline reached statistical significance only occasionally either in absolute or fractional terms (Table 2;

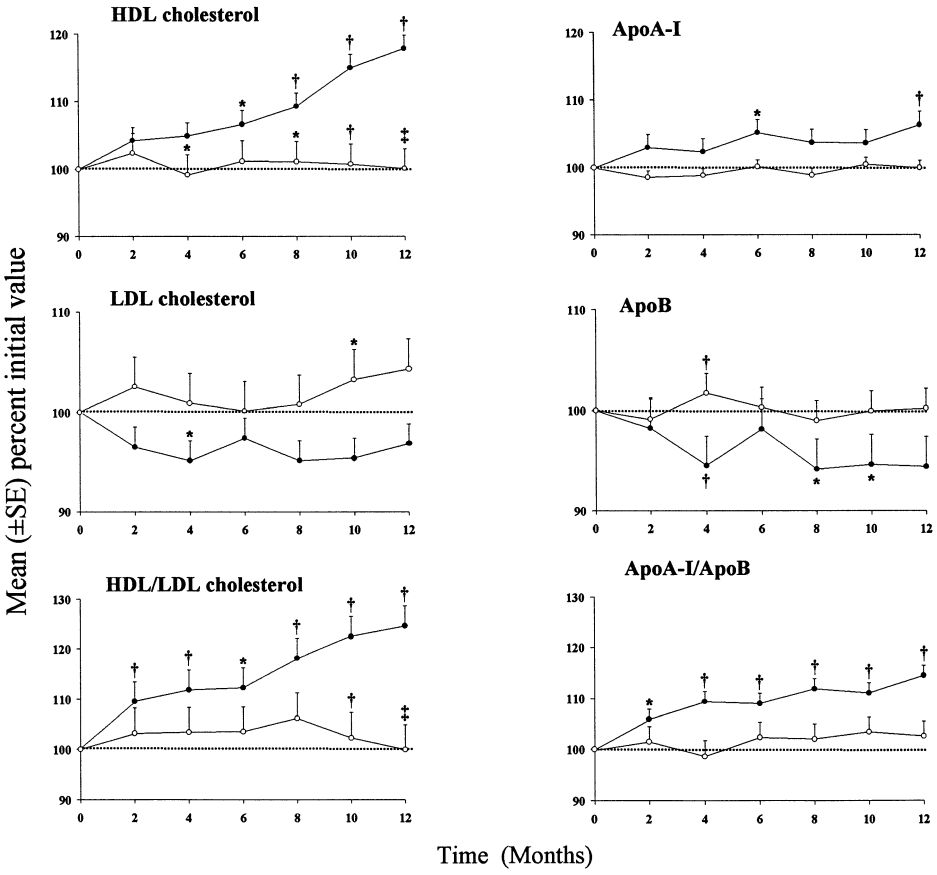

FIG. 3. Percent changes in serum total AP, bone AP, and f-DPD in Neridronate-treated (closed circles) and control patients (open circles). The symbols (*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$) over the closed circles indicate the statistical significance of the changes versus baseline in the Neridronate patients (Student's *t*-test for paired observation) and those over the open circles (control patients) indicate the between-group differences (ANOVA for repeated measures).

Fig. 1). Serum LDL-C decreased in the Neridronate-treated patients within 2 months by 4% and the difference was statistically significant at a few of the time points (Table 2; Fig. 2). Serum Apo B decreased also in the treated group by 6% and the changes paralleled those of LDL-C (Fig. 2). Serum HDL-C progressively rose during Neridronate therapy, from +4% ($p < 0.05$) after 2 months to +17–18% ($p < 0.0001$) in the 12th month. Serum Apo A-I also rose significantly at most time points in treated patients. The HDL-C/LDL-C ratio rose progressively up to +24% in the 12th month of Neridronate therapy. Significant increases in Apo A-I/Apo B ratio (Fig. 2) and significant decreases of total cholesterol/HDL-C (Table 2) also were noted in treated patients

DISCUSSION

Therapy with Neridronate resulted in significant reduction in serum concentration of LDL-C and Apo B and in remarkable increases in Apo A-I and HDL-C. The latter was still rising in the 12th month of observation. Similar trends were observed within 2 months in four patients (data not shown) given three consecutive daily doses of 60 mg Pamidronate iv (Aredia, Novartis, Italy; three patients) or of 7.5 mg Alendronate (Istituto Gentili, Italy; one patient), which are two aminobisphosphonates that structurally are strictly related to Neridronate. This indicates that the effect on lipoproteins pertains to all aminobisphosphonates. The lipid profile also was evaluated in 13 of the patients within 1–8 weeks after the first iv infusion of Neridronate as part of the routine safety assessment. In the six blood samples obtained in the fourth to sixth week, HDL-C was consistently increased. Actually, it also was this observation that led us to modify the research protocol and to keep serum aliquots for the lipid measurement.

The effects on the lipid profile of iv Neridronate appears to be cumulative for HDL-C lasting for at least 2 months after each dosing for all lipoproteins. This also was unexpected. For their pharmacodynamic features, bone cells have been considered the only possible target of bisphosphonates. In fact, these compounds concentrate at pharmacologically relevant concentrations only at the bone surfaces undergoing an active process of remodeling and the blood concentration achieved after oral administration is considered quite insufficient to lead to any pharmacologic effect on other cells (e.g., liver) involved in lipid metabolism.[10] Based on these assumptions when bisphosphonates are administered only iv, the blood concentrations rise high enough to interfere with cells that are different from those of the bone tissue, even though only with a very short lag time. In this study we have shown that the effects on lipid metabolism of aminobisphosphonates are long lasting despite the short half-life of both circulating bisphosphonates and circulating lipoproteins.[1,11] This suggests that the metabolic effects of amino-

TABLE 2. MEAN (±SE) VALUES OF SERUM LIPIDS IN NERIDRONATE AND CONTROL GROUP, BEFORE AND DURING FOLLOW-UP

	Therapy	*Baseline*	*2 months*	*4 months*	*6 months*	*8 months*	*10 months*	*12 months*
Cholesterol Mmol/liter (<5.17)	Neridronate	6.13 ± 0.18	5.93 ± 0.17	5.85 ± 0.16†	6.02 ± 0.16	5.86 ± 0.16	5.95 ± 0.16	6.08 ± 0.17
	Controls	5.76 ± 0.17	5.83 ± 0.16	5.74 ± 0.17	5.79 ± 0.18	5.86 ± 0.19	5.88 ± 0.18	5.86 ± 0.17
Triglycerides Mmol/liter (0.39–1.80)	Neridronate	1.51 ± 0.11	1.44 ± 0.09	1.35 ± 0.08	1.51 ± 0.11	1.35 ± 0.08*	1.37 ± 0.09	1.44 ± 0.12
	Controls	1.64 ± 0.11	1.68 ± 0.13	1.64 ± 0.11	1.69 ± 0.11	1.71 ± 0.14	1.71 ± 0.12	1.63 ± 0.11
HDL-C Mmol/liter (0.90–2.01)	Neridronate	1.09 ± 0.05	1.12 ± 0.05	1.13 ± 0.05	1.15 ± 0.05	1.17 ± 0.05†	1.23 ± 0.05‡	1.26 ± 0.05‡
	Controls	1.16 ± 0.05	1.18 ± 0.05	1.15 ± 0.05	1.16 ± 0.05	1.17 ± 0.05*	1.16 ± 0.06†	1.16 ± 0.06‡
LDL-C Mmol/liter (<3.74)	Neridronate	4.34 ± 0.17	4.14 ± 0.16	4.09 ± 0.15*	4.16 ± 0.15	4.06 ± 0.15*	4.08 ± 0.16*	4.15 ± 0.16
	Controls	3.84 ± 0.17	3.86 ± 0.17	3.83 ± 0.18	3.84 ± 0.18	3.89 ± 0.21	3.93 ± 0.19*	3.94 ± 0.18*
Total cholesterol/HDL-C ratio	Neridronate	6.20 ± 0.35	5.78 ± 0.34*	5.68 ± 0.33‡	5.72 ± 0.30	5.44 ± 0.30‡	5.26 ± 0.27‡	5.21 ± 0.26‡
	Controls	5.35 ± 0.28	5.33 ± 0.29*	5.55 ± 0.37†	5.37 ± 0.28	5.67 ± 0.40‡	6.15 ± 0.72*	5.85 ± 0.43‡
Apo A-I g/liter (>1.1)	Neridronate	1.69 ± 0.04	1.73 ± 0.04	1.72 ± 0.04	1.78 ± 0.05*	1.75 ± 0.04	1.74 ± 0.04	1.79 ± 0.04†
	Controls	1.65 ± 0.04	1.63 ± 0.04	1.64 ± 0.04	1.66 ± 0.04*	1.64 ± 0.04*	1.66 ± 0.04	1.66 ± 0.04†
Apo B g/liter (<1.5)	Neridronate	1.37 ± 0.05	1.34 ± 0.06	1.29 ± 0.06*	1.33 ± 0.05	1.28 ± 0.06*	1.28 ± 0.05*	1.28 ± 0.06*
	Controls	1.35 ± 0.05	1.33 ± 0.05	1.37 ± 0.04†	1.36 ± 0.05	1.34 ± 0.05	1.35 ± 0.05	1.36 ± 0.05

The significance symbols on the Neridronate data refer to the changes versus baseline (paired *t*-test). The significance symbols on the control data refer to the differences versus the Neridronate group.

*$p < 0.05$; †$p < 0.01$; ‡$p < 0.001$.

bisphosphonates might not be attributed exclusively to the simple inhibition of enzymes involved in cholesterol synthesis.[4,5]

The changes in circulating lipoproteins are not accompanied by relevant changes in total serum cholesterol and in serum triglycerides and this might explain why the remarkable effects on lipid profile observed here have never been detected in previous studies, within the routine safety assessment, which rarely include the evaluation of circulating lipoproteins. We also are unaware of any such findings in animal studies, even though for some of them (e.g., Alendronate) a thorough investigation of lipid profile was not included (Dr. Gideon Rodan, personal communication).

Given the knowledge of the mechanism of action of bisphosphonates, we are unable to explain the observed pattern of changes in lipoproteins, which basically consists in a molar shift in circulating cholesterol from LDL to HDL forms. The decrease in serum LDL-C and in serum Apo B is lower than that observed during hormone replacement therapy in postmenopausal women or during statin administration.[12–15] However, the magnitude of the increases in serum HDL-C and Apo A-I and the HDL/LDL-C ratio after 1 year of intermittent iv Neridronate are clinically relevant and apparently favorable. The results of this study indicate that aminobisphosphonates given iv may interfere with lipid metabolism. This finding has clinical implications because some iv aminobisphosphonates (e.g., Pamidronate) are used extensively for the treatment of Paget's disease and for bone malignancies and others (e.g., Ibandronate) are expected to be registered with the iv intermittent formulation for the treatment of postmenopausal osteoporosis.

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