Appendix 1

Study of Women's Health Across the Nation (SWAN)
Body Composition by Dual Energy X-Ray Absorptiometry (DXA)

Standard Protocols, Hardware and Software Versions, and Cross Calibration
Baseline through SWAN Follow-Up Visit 13

A. Summary of SWAN Body Composition Participant Measurement and Analysis Protocol and Management of Metal Objects

Bone density and body composition scans were done with participants wearing only undergarments and a gown, scrubs, or a sheet. Participant positioning on the scan bed was according to the manufacturer's standard protocol (Hologic, Inc., Waltham, MA). If a participant was too large to permit proper spacing between the legs and torso, then her left arm was intentionally placed out of the scan field (to allow proper spacing of the remainder of the body).

Participants were asked to remove metal or jade objects, which interfere with bone mineral content (BMC) readings. Un-removable metal below the neck (or items that the participants refused to or could not remove) were recorded and classified using a study wide, standardized system. Un-removable metal classifications were "trivial", such as a ring or navel stud, or "non-trivial" such as a joint replacement. We necessarily excluded scans that contained non-trivial metal from total body bone density (TBBMD) readings. We computed whole body lean mass computations without BMC to obviate contamination by metal or jade objects.

Technologists analyzed TBBMD and body composition according to the manufacturer's standard instructions (Hologic, Inc., Waltham, MA). Instructions for placing the lines optimally for TBBMD sometimes results in incorrect separation of the soft tissue regions for body composition. When the correct definition of skeletal sub-regions prevented the correct definition

of soft tissue sub-regions) technologists conducted two separate analyses, one that defined TBBMD correctly and one that defined regions for body composition correctly; the two analyses were saved as separate files.

B. Cross-Calibration of Body Composition Readings from Hologic QDR-2000 Model to QDR-4500 or Discovery Model at UC Davis/Kaiser and University of Pittsburgh Sites

Densitometer changes at the UC Davis/Kaiser and University of Pittsburgh SWAN Sites.

The UC Davis/Kaiser and University of Pittsburgh SWAN sites initially acquired body composition scans using a Hologic 2000 densitometer. At the start of follow-up visit 8 (March, 2004), UC Davis/Kaiser switched to a Hologic Discovery and Pittsburgh to a Hologic 4500. To prepare for this hardware change, each site conducted a human cross-calibration study. Volunteer women (N=33 at Davis/Kaiser, N=40 at Pittsburgh, with a mean age of 58 and of varying BMI) underwent two DXA scans within a maximum of 3 months, first on the Hologic 2000 and second on the newer machine. As expected, there were systematic differences between body composition readings obtained with the older vs. newer machines: newer models read-out less fat and more lean tissue than does the 2000 model (Table 1). Hologic developed body composition conversion equations for its hardware and software that apply to the 4500 machine and those that followed it [1]. However, they did not create conversions for body composition values obtained on the 2000 model to make them compatible with later models. We, therefore, capitalized on our human cross-calibration studies to create conversion equations for the 2000 to the 4500 and to the Discovery models. Investigators have done similar human cross calibrations between Hologic and Lunar instruments [2].

Table 1. Differences in lean and fat mass values based on readings from original hardware (Hologic 2000) and new hardware (Discovery at Davis/Kaiser and 4500 at Pittsburgh) [a,b,c]

	Davis/Kaiser Site (n=33)	Pittsburgh Site (n=40)
	Mean (SD)	Mean (SD)
Total Lean, grams	5.64 (3.52)	3.80 (1.95)
Total Fat, grams	-3.65 (2.99)	-3.40 (2.02)
Percent Fat	-5.78 (3.16)	-4.66 (1.78)

[a] Total lean and fat values reported do not include the head.
[b] Differences are computed as (value from newer hardware – value from older hardware)
[c] Median differences similar to mean differences

Calibrations for Hologic 2000 to 4500 and Hologic 2000 to Discovery. We created calibration equations using SWAN's human cross-calibration scans as described below. The first step was to apply the NHANES-Apex tissue calibration to the 4500 or Discovery values (using the NHANES-Apex metric provided by Hologic, Inc.) (1). Here, total lean2 refers to lean mass without bone mineral content (BMC). We use lean2 to avoid contamination by non-removable metal, such as joint replacements, which falsely elevate BMC and therefore falsely lower non-bone lean tissue mass).

$$Total\ fat_{NHANES} = Total\ fat + 0.054 * Total\ lean2$$

$$Total\ lean2_{NHANES} = Total\ lean2 - 0.054 * Total\ lean2$$

Next, we plotted the within-woman, between-machine differences of values obtained with each site's new and old DXA machines against the average of the two (Bland-Altman plots) [3]. The plots revealed a clear, linear trend: the difference increased (or decreased) as the average increased. Based on these linear trends, we regressed the between-machine difference on the average over the two machines, to create calibration equations for each SWAN site. In separate models, we regressed each of the fat and lean variables on: a) both fat and lean2; b) fat only; and c) lean2 only. We summarize principal findings from these regressions below:

1. <u>For all outcomes, regression on fat alone was just as good as regression on fat and lean2</u>. Regression on lean2 alone did not perform well. For example, in the Pittsburgh

sample, the R^2 for between-machine difference in total fat was 0.92 for prediction by total fat alone and 0.93 for prediction by combination of total fat and total lean2 (likewise, 0.80 and 0.80 in the Davis/Kaiser sample); the R^2 for between-machine difference in total lean2 was 0.87 for prediction by total fat alone and 0.89 for prediction by combination of total fat and total lean2 (likewise, 0.85 and 0.85 in the Davis/Kaiser sample).

2. SWAN site-specific R^2 for total fat and lean2 values were good (0.8 or better) but R^2 dropped dramatically when data from the 2 clinical sites were combined. This indicates that that the calibration equations must be specific to the clinical site for fidelity. For example, the R^2 for predicting total lean2 was only 0.48 in the combined data.

SWAN Site Specific Calibrations for Pittsburgh and Davis/Kaiser. Using the methods described thus far, we developed the following site specific calibrations to convert QDR-2000 data to equivalent QDR -4500 (Pittsburgh) or Discovery (Davis/Kaiser) data.

Pittsburgh

Total fat and lean without BMC (lean2):

$$Total\ fat_{calibrated} = 2744 + 0.8645 * Total\ fat_{QDR-2000}$$

(calibration error variance = 0.2%)

$$Total\ lean2_{calibrated} = -2180 + Total\ lean2_{QDR-2000} + 0.1282 * Total\ fat_{QDR-2000}$$

(calibration error variance = 2.2%)

UC Davis/Kaiser

Total fat and lean without BMC (lean2):

$$Total\ fat_{calibrated} = 3140 + 0.8252 * Total\ fat_{QDR-2000}$$

(calibration error variance = 0.9%)

$$Total\ lean2_{calibrated} = -2372 + Total\ lean2_{QDR-2000} + 0.2186 * Total\ fat_{QDR-2000}$$

(calibration error variance = 3.9%)

C. Cross-Calibration of Body Composition Readings from QDR-4500 to Discovery Model at Massachusetts General Hospital (MGH) and UCLA sites

The MGH SWAN site switched from Hologic 4500 to Discovery in January of 2010. The UCLA SWAN site changed from Hologic 4500 to Discovery in April of 2012. As noted above, Hologic created fat mass and lean mass conversion equations to calibrate data collected with its 4500 machines to that obtained with subsequently released hardware [1]. However, rather than just applying the Hologic-developed fat and lean calibrations, we also investigated whether the hardware changes themselves required additional calibration. To test the latter question, we used data from human cross-calibration scans. Shown in **Table 2** are sizes of cross-calibration samples and the tissue referents that were running on each machine.

Table 2. Summary of DXA machine changes, human cross-calibration sample sizes, and the software and tissue referents used during cross-calibration scans at MGH and UCLA SWAN sites

Site	Sample Size	1st cross calibration scan (4500)			2nd cross calibration scan (Discovery)		
		Machine	Software	Tissue Bar	Machine	Software	Tissue Bar
MGH	23	4500	Version 12.4	Classic	Discovery A	Version 13.0.1	NHANES Pre-APEX
		1st cross calibration scan (4500)			2nd cross calibration scan (Discovery)		
		Machine	Software	Tissue Bar	Machine	Software	Tissue Bar
UCLA	37	4500	Version 12.1.3	Classic	Discovery A	Version 13.3	NHANES Pre-APEX

The first step was to convert all SWAN body composition scans to the NHANES Apex tissue standard using the equations provided by Hologic and reproduced below [1]. In these equations, lean soft tissue [classic] = lean mass without BMC.

In brief, between 1995 and 2013, Hologic calculated whole body composition three different ways:

- Classic Hologic software configuration
- NHANES Pre-APEX 3.4 Hologic software configuration
- NHANES APEX Hologic software configuration

The three methods give different results for whole body fat and lean mass, but similar results for whole body bone mineral and whole body BMD.

<u>To convert to NHANES Apex from Classic:</u>

$$Fat_{NHANES\ Apex} = Fat_{Classic} + 0.054 * \ Lean\ soft\ tissue_{classic}$$

$$Lean\ soft\ tissue_{NHANES\ Apex}$$

$$= Total\ Mass_{NHANES\ Apex} \ - \ Fat_{NHANES\ Apex} - BMC_{NHANES\ Apex}$$

$$= Total\ Mass_{classic} - (Fat_{classic} + 0.054 * Lean\ soft\ tissue_{classic}) - BMC_{classic}$$

$$= Total\ Mass_{classic} - Fat_{classic} - 0.054 * Lean\ soft\ tissue_{classic} - BMC_{classic}$$

<u>To convert to NHANES APEX from NHANES Pre-Apex 3.4:</u>

$$Fat_{NHANES\ Apex} = Fat_{NHANES\ Pre-Apex} - 0.054 * \ BMC_{NHANES\ Pre-Apex}$$

$$Lean\ soft\ tissue_{NHANES\ Apex}$$

$$= Total\ Mass_{NHANES\ Apex} \ - \ Fat_{NHANES\ Apex} - BMC_{NHANES\ Apex}$$

$$= Total\ Mass_{NHANES\ Apex} \ - \left(Fat_{NHANES\ Pre-Apex} - 0.054 * BMC_{NHANES\ Pre-Apex}\right) - BMC_{NHANES\ Apex}$$

$$= Total\ Mass_{NHANES\ Pre-Apex} \ - Fat_{NHANES\ Pre-Apex} - 0.946 * \ BMC_{NHANES\ Pre-Apex}$$

Calibration Equations to convert Discovery data to equivalent QDR-4500 data (using NHANES APEX tissue type) at MGH. Discovery total fat and total lean2 (without BMC) do not require any further conversion beyond application of the Hologic equations. That is, there was no unique influence of the hardware change at MGH.

Calibration Equations to convert Discovery data to equivalent QDR-4500 data (using NHANES APEX tissue type) at UCLA. Discovery total lean2 (without BMC) did not require further conversion beyond application of the Hologic equation. The total fat values from Discovery machine did require a small calibration to match QDR-4500 data (i.e., there was a unique influence of the hardware change). The calibration equation for UCLA total fat is:

$$Total\ fat_{QDR-4500} = 0.985 * Total\ fat_{Discovery} - 725$$

D. Precision Estimates for Body Composition Measures

Adapted from a Hologic, Inc., technical paper prepared in December 2010 by Thomas L. Kelly, Principal Scientist. Data for the 4500A and Discovery A instruments were acquired using fan beam technology.

Table 3. Precision estimates for DXA body composition measures using selected Hologic Inc., instruments[1]

First Author of Reference	QDR Model	BMC (g)	Fat Mass (g)	Lean Mass (g)	Total Mass	Percent Fat
Chilibeck [4]	2000	1.6%	1.8%	1.4%		
Abrahamsen [5]	2000		1.9% (280 g)	0.6% (310 g)		
Fuerst [6]	4500A		2.1% (450 g)	1.0% (430 g)		1.0%
K. Univ., [7]	4500A	0.7% (15 g)	1.5% (245 g)	0.4% (190 g)	0.2% (146 g)	1.3% (0.3 g)
CM Leonard [8] (Children < 10 yrs.)	Discovery A V12.3	1.3% (12.9 g)	2.2% (172 g)	0.9% (201 g)		
CM Leonard [8] (Children 10-18 yrs.)	Discovery A V12.3	1.2% (17.6 g)	1.9% (189 g)	0.7% (251 g)		

[1] Numbers in brackets refer to citation from which the data are abstracted (see References).

References

1. Hologic Advanced Body Composition Analysis and NHANES Calibration, February 20, 2013.

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